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| (21) International Application Number: PCT/US98/01507 (22) International Filing Date: 27 January 1998 (27.01.98) (30) Priority Data: 60/036,048 27 January 1997 (27.01.97) US (71) Applicant: CORNELL RESEARCH FOUNDATION, INC. [US/US]; Suite 105, 20 Thornwood Drive, Ithaca, NY 14850 (US). (72) Inventors: QIU, Dewen; 17815 8th Avenue, N.E., Seattle, WA 98155 (US). WEI, Zhong-Min; 8230 N.E. 125th Court, Kirkland, WA 98034 (US). BEER, Steven, V.; 211 Hudson Street, Ithaca, NY 14850 (US). (74) Agents: GOLDMAN, Michael, L. et al.; Nixon, Hargrave, Devans & Doyle LLP, Clinton Square, P.O. Box 1051, Rochester, NY 14603 (US). | | | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> |
| (54) Title: ENHANCEMENT OF GROWTH IN PLANTS | | | |
| (57) Abstract <p>The present invention relates to a method of enhancing growth of plants. This involves applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to a plant or plant seed under conditions effective to enhance growth of the plant or plants produced from the plant seed. Alternatively, transgenic plants or transgenic plant seeds transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to enhance plant growth.</p> | | | |

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ENHANCEMENT OF GROWTH IN PLANTS

This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/036,048, filed January 27, 1997.

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5 U.S. Government under USDA NRI Competitive Research Grant No. 91-37303-6430.

FIELD OF THE INVENTION

10 The present invention relates to the enhancement of growth in plants.

BACKGROUND OF THE INVENTION

15 The improvement of plant growth by the application of organic fertilizers has been known and carried out for centuries (H. Marschner, "Mineral Nutrition of Higher Plants," Academic Press: New York pg. 674 (1986)). Modern man has developed a complex
20 inorganic fertilizer production system to produce an easy product that growers and farmers can apply to soils or growing crops to improve performance by way of growth enhancement. Plant size, coloration, maturation, and yield may all be improved by the application of
25 fertilizer products. Inorganic fertilizers include such commonly applied chemicals as ammonium nitrate. Organic fertilizers may include animal manures and composted lawn debris, among many other sources.

In most recent years, researchers have sought
30 to improve plant growth through the use of biological products. Insect and disease control agents such as *Beauveria bassiana* and *Trichoderma harizamum* have been registered for the control of insect and disease problems and thereby indirectly improve plant growth and
35 performance (Fravel et al., "Formulation of

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Microorganisms to Control Plant Diseases," Formulation of Microbial Biopesticides, Beneficial Microorganisms, and Nematodes, H.D. Burges, ed. Chapman and Hall: London (1996).

5 There is some indication of direct plant growth enhancement by way of microbial application or microbial by-products. Nodulating bacteria have been added to seeds of leguminous crops when introduced to a new site (Weaver et al., "Rhizobium," Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, 2nd ed., 10 American Society of Agronomy: Madison (1982)). These bacteria may improve the nodulation efficiency of the plant and thereby improve the plant's ability to convert free nitrogen into a usable form, a process called 15 nitrogen fixation. Non-leguminous crops do not, as a rule, benefit from such treatment. Added bacteria such as *Rhizobium* directly parasitize the root hairs, then begin a mutualistic relationship by providing benefit to the plant while receiving protection and sustenance.

20 Mycorrhizal fungi have also been recognized as necessary microorganisms for optional growth of many crops, especially conifers in nutrient-depleted soils. Mechanisms including biosynthesis of plant hormones (Frankenberger et al., "Biosynthesis of Indole-3-Acetic 25 Acid by the Pine Ectomycorrhizal Fungus *Pisolithus tinctorius*," Appl. Environ. Microbiol. 53:2908-13 (1987)), increased uptake of minerals (Harley et al., "The Uptake of Phosphate by Excised Mycorrhizal Roots of Beech," New Phytologist 49:388-97 (1950) and Harley et 30 al., "The Uptake of Phosphate by Excised Mycorrhizal Roots of Beech. IV. The Effect of Oxygen Concentration Upon Host and Fungus," New Phytologist 52:124-32 (1953)), and water (A.B. Hatch, "The Physical Basis of Mycotrophy in *Pinus*," Black Rock Forest Bull. No. 6, 168 pp. (1937)) 35 have been postulated. Mycorrhizal fungi have not

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achieved the common frequency of use that nodulating bacteria have due to variable and inconsistent results with any given mycorrhizal strain and the difficulty of study of the organisms.

- 5 Plant growth-promoting rhizobacteria ("PGPR") have been recognized in recent years for improving plant growth and development. Hypothetical mechanisms range from direct influences (e.g., increased nutrient uptake) to indirect mechanisms (e.g., pathogen displacement).
- 10 Growth enhancement by application of a PGPR generally refers to inoculation with a live bacterium to the root system and achieving improved growth through bacterium-produced hormonal effects, siderophores, or by prevention of disease through antibiotic production, or competition.
- 15 In all of the above cases, the result is effected through root colonization, sometimes through the application of seed coatings. There is limited information to suggest that some PGPR strains may be direct growth promoters that enhance root elongation under gnotobiotic conditions
- 20 (Anderson et al., "Responses of Bean to Root Colonization With *Pseudomonas putida* in a Hydroponic System," Phytopathology 75:992-95 (1985), Lifshitz et al., "Growth Promotion of Canola (rapeseed) Seedlings by a Strain of *Pseudomonas putida* Under Gnotobiotic Conditions," Can. J. Microbiol. 33:390-95 (1987), Young et al., "PGPR: Is
- 25 There Relationship Between Plant Growth Regulators and the Stimulation of Plant Growth or Biological Activity?," Promoting Rhizobacteria: Progress and Prospects, Second International Workshop on Plant Growth-promoting
- 30 Rhizobacteria, pp. 182-86 (1991), Loper et al., "Influence of Bacterial Sources of Indole-3-Acetic Acid on Root Elongation of Sugar Beet," Phytopathology 76:386-89 (1986), and Müller et al., "Hormonal Interactions in the Rhizosphere of Maize (*Zea mays* L.) and Their Effect
- 35 on Plant Development," Z. Pflanzenernährung Bodenkunde

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152:247-54 (1989); however, the production of plant growth regulators has been proposed as the mechanism mediating these effects. Many bacteria produce various plant growth regulators in vitro (Atzorn et al.,

5 "Production of Gibberellins and Indole-3-Acetic Acid by *Rhizobium phaseoli* in Relation to Nodulation of *Phaseolus vulgaris* Roots," Planta 175:532-38 (1988) and M. E. Brown, "Plant Growth Substances Produced by Micro-

10 Organism of Solid and Rhizosphere," J. Appl. Bact. 35:443-51 (1972)) or antibiotics (Gardner et al., "Growth Promotion and Inhibition by Antibiotic-Producing Fluorescent *Pseudomonads* on Citrus Roots," Plant Soil 77:103-13 (1984)). Siderophore production is another mechanism proposed for some PGPR strains (Ahl et al.,

15 "Iron Bound-Siderophores, Cyanic Acid, and Antibiotics Involved in Suppression of *Thiaveliopsis basicola* by a *Pseudomonas fluorescens* Strain," J. Phytopathol. 116:121-34 (1986), Kloepper et al., "Enhanced Plant Growth by Siderophores Produced by Plant Growth-Promoting

20 Rhizobacteria," Nature 286:885-86 (1980), and Kloepper et al., "Pseudomonas siderophores: A Mechanism Explaining Disease-Suppressive Soils," Curr. Microbiol. 4:317-20 (1980)). The colonization of root surfaces and thus the direct competition with pathogenic bacteria on the

25 surfaces is another mechanism of action (Kloepper et al., "Relationship of in vitro Antibiosis of Plant Growth-Promoting Rhizobacteria to Plant Growth and the Displacement of Root Microflora," Phytopathology 71:1020-24 (1981), Weller, et al., "Increased Growth of Wheat by

30 Seed Treatments With Fluorescent *Pseudomonads*, and Implications of *Pythium* Control," Can. J. Microbiol. 8:328-34 (1986), and Suslow et al., "Rhizobacteria of Sugar Beets: Effects of Seed Application and Root Colonization on Yield," Phytopathology 72:199-206

35 (1982)). Canola (rapeseed) studies have indicated PGPR

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increased plant growth parameters including yields, seedling emergence and vigor, early-season plant growth (number of leaves and length of main runner), and leaf area (Kloepper et al., "Plant Growth-Promoting Rhizobacteria on Canola (rapeseed)," Plant Disease 72:42-46 (1988)). Studies with potato indicated greater yields when *Pseudomonas* strains were applied to seed potatoes (Burr et al., "Increased Potato Yields by Treatment of Seed Pieces With Specific Strains of *Pseudomonas* *Fluorescens* and *P. putida*," Phytopathology 68:1377-83 (1978), Kloepper et al., "Effect of Seed Piece Inoculation With Plant Growth-Promoting Rhizobacteria on Populations of *Erwinia carotovora* on Potato Roots and in Daughter Tubers," Phytopathology 73:217-19 (1983), Geels et al., "Reduction of Yield Depressions in High Frequency Potato Cropping Soil After Seed Tuber Treatments With Antagonistic Fluorescent *Pseudomonas* spp.," Phytopathol. Z. 108:207-38 (1983), Howie et al., "Rhizobacteria: Influence of Cultivar and Soil Type on Plant Growth and Yield of Potato," Soil Biol. Biochem. 15:127-32 (1983), and Vransy et al., "Growth and Yield of Potato Plants Inoculated With Rhizosphere Bacteria," Folia Microbiol. 29:248-53 (1984)). Yield increase was apparently due to the competitive effects of the PGPR to eliminate pathogenic bacteria on the seed tuber, possibly by antibiosis (Kloepper et al., "Effect of Seed Piece Inoculation With Plant Growth-Promoting Rhizobacteria on Populations of *Erwinia carotovora* on Potato Roots and in Daughter Tubers," Phytopathology 73:217-19 (1983), Kloepper et al., "Effects of Rhizosphere Colonization by Plant Growth-Promoting Rhizobacteria on Potato Plant Development and Yield," Phytopathology 70:1078-82 (1980), Kloepper et al., "Emergence-Promoting Rhizobacteria: Description and Implications for Agriculture," pp. 155-164, Iron, Siderophores, and Plant Disease, T.R.

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Swinburne, ed. Plenum, New York (1986), and Kloepper et al., "Relationship of in vitro Antibiosis of Plant Growth-Promoting Rhizobacteria to Plant Growth and the Displacement of Root Microflora," Phytopathology 71:1020-24 (1981)). In several studies, plant emergence was improved using PGPR (Tipping et al., "Development of Emergence-Promoting Rhizobacteria for Supersweet Corn," Phytopathology 76:938-41 (1990) (abstract) and Kloepper et al., "Emergence-Promoting Rhizobacteria: Description and Implications for Agriculture," pp. 155-164, Iron, Siderophores, and Plant Disease, T.R. Swinburne, ed. Plenum, New York (1986)). Numerous other studies indicated improved plant health upon treatment with rhizobacteria, due to biocontrol of plant pathogens (B. Schippers, "Biological Control of Pathogens With Rhizobacteria," Phil. Trans. R. Soc. Lond. B. 318:283-93 (1988), Schroth et al., "Disease-Suppressive Soil and Root-Colonizing Bacteria," Science 216:1376-81 (1982), Stutz et al., "Naturally Occurring Fluorescent Pseudomonads Involved in Suppression of Black Root Rot of Tobacco," Phytopathology 76:181-85 (1986), and D.M. Weller, "Biological Control of Soilborne Plant Pathogens in the Rhizosphere With Bacteria," Annu. Rev. Phytopathol. 26:379-407 (1988)).

Pathogen-induced immunization of a plant has been found to promote growth. Injection of *Peronospora tabacina* externally to tobacco xylem not only alleviated stunting but also promoted growth and development. Immunized tobacco plants, in both greenhouse and field experiments, were approximately 40% taller, had a 40% increase in dry weight, a 30% increase in fresh weight, and 4-6 more leaves than control plants (Tuzun, S., et al., "The Effect of Stem Injection with *Peronospora tabacina* and Metalaxyl Treatment on Growth of Tobacco and Protection Against Blue Mould in the Field,"

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Phytopathology, 74:804 (1984). These plants flowered approximately 2-3 weeks earlier than control plants (Tuzun, S., et al., "Movement of a Factor in Tobacco Infected with *Peronospora tabacina* Adam which Systemically Protects Against Blue Mould," Physiological Plant Pathology, 26:321-30 (1985)).

The present invention is directed to an improvement over prior plant growth enhancement procedures.

10

SUMMARY OF THE INVENTION

The present invention relates to a method of enhancing growth in plants. This method involves applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to plants or plant seeds under conditions to impart enhanced growth to the plants or to plants grown from the plant seeds.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart enhanced growth to the plants or to plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to enhance growth. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to enhance growth.

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The present invention is directed to effecting any form of plant growth enhancement or promotion. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land. It is thus apparent that the present invention constitutes a significant advance in agricultural efficiency.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a map of plasmid vector pCPP2139 which contains the *Erwinia amylovora* hypersensitive response elicitor gene.

Figure 2 is a map of plasmid vector pCPP50 which does not contain the *Erwinia amylovora* hypersensitive response elicitor gene but is otherwise the same as plasmid vector pCPP2139 shown in Figure 1. See Masui, et al., Bio/Technology 2:81-85 (1984), which is hereby incorporated by reference.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of enhancing growth in plants. This method involves
5 applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed under conditions to impart enhanced growth to the plant or to a plant grown from the plant seed. Alternatively, plants can be treated in this
10 manner to produce seeds, which when planted, impart enhanced growth in progeny plants.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart enhanced growth to the
15 plants or to plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein
20 and growing the plant under conditions effective to permit that DNA molecule to enhance growth. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in
25 soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to enhance growth.

The hypersensitive response elicitor polypeptide or protein utilized in the present invention
30 can correspond to hypersensitive response elicitor polypeptides or proteins derived from a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor.

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Examples of suitable bacterial sources of polypeptide or protein elicitors include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*,
5 *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof).

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide
10 is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora pythium*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

The embodiment of the present invention where
15 the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated elicitor polypeptide or protein; 2) application of bacteria which do not cause
20 disease and are transformed with genes encoding a hypersensitive response elicitor polypeptide or protein; and 3) application of bacteria which cause disease in some plant species (but not in those to which they are applied) and naturally contain a gene encoding the
25 hypersensitive response elicitor polypeptide or protein. In addition, seeds in accordance with the present invention can be recovered from plants which have been treated with a hypersensitive response elicitor protein or polypeptide in accordance with the present invention.

30 In one embodiment of the present invention, the hypersensitive response elicitor polypeptides or proteins can be isolated from their corresponding organisms and applied to plants or plant seeds. Such isolation procedures are well known, as described in
35 Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet,

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and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994); He, S. Y., H. C. Huang, and A. Collmer, "*Pseudomonas syringae* pv. *syringae* Harpin_{pss}: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993); and Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which are hereby incorporated by reference. See also pending U.S. Patent Application Serial Nos. 08/200,024 and 08/062,024, which are hereby incorporated by reference. Preferably, however, the isolated hypersensitive response elicitor polypeptides or proteins of the present invention are produced recombinantly and purified as described below.

In other embodiments of the present invention, the hypersensitive response elicitor polypeptide or protein of the present invention can be applied to plants or plant seeds by applying bacteria containing genes encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the polypeptide or protein so that the elicitor can contact plant or plant seeds cells. In these embodiments, the hypersensitive response elicitor polypeptide or protein is produced by the bacteria in planta or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

In one embodiment of the bacterial application mode of the present invention, the bacteria do not cause the disease and have been transformed (e.g., recombinantly) with genes encoding a hypersensitive response elicitor polypeptide or protein. For example,

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E. coli, which does not elicit a hypersensitive response in plants, can be transformed with genes encoding a hypersensitive response elicitor polypeptide or protein and then applied to plants. Bacterial species other than
 5 *E. coli* can also be used in this embodiment of the present invention.

In another embodiment of the bacterial application mode of the present invention, the bacteria do cause disease and naturally contain a gene encoding a
 10 hypersensitive response elicitor polypeptide or protein. Examples of such bacteria are noted above. However, in this embodiment, these bacteria are applied to plants or their seeds which are not susceptible to the disease carried by the bacteria. For example, *Erwinia amylovora*
 15 causes disease in apple or pear but not in tomato. However, such bacteria will elicit a hypersensitive response in tomato. Accordingly, in accordance with this embodiment of the present invention, *Erwinia amylovora* can be applied to tomato plants or seeds to enhance
 20 growth without causing disease in that species.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

25

Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
 1 5 10 15

30

Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
 20 25 30

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
 35 40 45

35

Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
 50 55 60

40

Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
 65 70 75 80

Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
 85 90 95

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|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | Ser | Gly | Gly | Asp | Ala | Leu | Ser | Lys | Met | Phe | Asp | Lys | Ala | Leu | Asp | Asp |
| | | | | 100 | | | | | 105 | | | | | 110 | | |
| 5 | Leu | Leu | Gly | His | Asp | Thr | Val | Thr | Lys | Leu | Thr | Asn | Gln | Ser | Asn | Gln |
| | | | 115 | | | | | 120 | | | | | 125 | | | |
| | Leu | Ala | Asn | Ser | Met | Leu | Asn | Ala | Ser | Gln | Met | Thr | Gln | Gly | Asn | Met |
| | | 130 | | | | | 135 | | | | | 140 | | | | |
| 10 | Asn | Ala | Phe | Gly | Ser | Gly | Val | Asn | Asn | Ala | Leu | Ser | Ser | Ile | Leu | Gly |
| | 145 | | | | | 150 | | | | | 155 | | | | | 160 |
| | Asn | Gly | Leu | Gly | Gln | Ser | Met | Ser | Gly | Phe | Ser | Gln | Pro | Ser | Leu | Gly |
| | | | | 165 | | | | | | 170 | | | | | 175 | |
| 15 | Ala | Gly | Gly | Leu | Gln | Gly | Leu | Ser | Gly | Ala | Gly | Ala | Phe | Asn | Gln | Leu |
| | | | | 180 | | | | | 185 | | | | | 190 | | |
| 20 | Gly | Asn | Ala | Ile | Gly | Met | Gly | Val | Gly | Gln | Asn | Ala | Ala | Leu | Ser | Ala |
| | | | 195 | | | | | 200 | | | | | 205 | | | |
| | Leu | Ser | Asn | Val | Ser | Thr | His | Val | Asp | Gly | Asn | Asn | Arg | His | Phe | Val |
| | | 210 | | | | | 215 | | | | | 220 | | | | |
| 25 | Asp | Lys | Glu | Asp | Arg | Gly | Met | Ala | Lys | Glu | Ile | Gly | Gln | Phe | Met | Asp |
| | 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| | Gln | Tyr | Pro | Glu | Ile | Phe | Gly | Lys | Pro | Glu | Tyr | Gln | Lys | Asp | Gly | Trp |
| | | | | | 245 | | | | | 250 | | | | | 255 | |
| 30 | Ser | Ser | Pro | Lys | Thr | Asp | Asp | Lys | Ser | Trp | Ala | Lys | Ala | Leu | Ser | Lys |
| | | | | 260 | | | | | 265 | | | | | 270 | | |
| 35 | Pro | Asp | Asp | Asp | Gly | Met | Thr | Gly | Ala | Ser | Met | Asp | Lys | Phe | Arg | Gln |
| | | | 275 | | | | | 280 | | | | | 285 | | | |
| | Ala | Met | Gly | Met | Ile | Lys | Ser | Ala | Val | Ala | Gly | Asp | Thr | Gly | Asn | Thr |
| | | 290 | | | | | 295 | | | | | 300 | | | | |
| 40 | Asn | Leu | Asn | Leu | Arg | Gly | Ala | Gly | Gly | Ala | Ser | Leu | Gly | Ile | Asp | Ala |
| | 305 | | | | | 310 | | | | | 315 | | | | | 320 |
| | Ala | Val | Val | Gly | Asp | Lys | Ile | Ala | Asn | Met | Ser | Leu | Gly | Lys | Leu | Ala |
| | | | | | 325 | | | | | 330 | | | | | 335 | |
| 45 | Asn | Ala | | | | | | | | | | | | | | |

This hypersensitive response elicitor polypeptide or
 50 protein has a molecular weight of 34 kDa, is heat stable,
 has a glycine content of greater than 16%, and contains
 substantially no cysteine. The *Erwinia chrysanthemi*
 hypersensitive response elicitor polypeptide or protein
 is encoded by a DNA molecule having a nucleotide sequence
 55 corresponding to SEQ. ID. No. 2 as follows:

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| | | |
|----|--|------|
| | CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTCGA CACCGTTACG | 60 |
| | GCGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTGCGCCGA ATCCGGCGTC | 120 |
| 5 | GATCTGGTAT TTCAGTTTGG GGACACCGGG CGTGAACCTCA TGATGCAGAT TCAGCCGGGG | 180 |
| | CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GGCGGCAGAG | 240 |
| | TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG | 300 |
| 10 | CCGTGCGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTTGAACCT GGCGGGAATG | 360 |
| | ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CGGACGCGCC | 420 |
| 15 | CGATCATTAA GATAAAGGCG GCTTTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT | 480 |
| | CACCGTCGGC GTCACCTCAGT AACAACTATC CATCATGATG CCTACATCGG GATCGGCGTG | 540 |
| | GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA | 600 |
| 20 | AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGGCGTC TCCGGTCTGG GGCTGGGTGC | 660 |
| | TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTTCAGCG TGGATAAACT | 720 |
| 25 | GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGCGCT | 780 |
| | GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC | 840 |
| | TTTCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCGA | 900 |
| 30 | TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC | 960 |
| | CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC | 1020 |
| 35 | CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG | 1080 |
| | CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT | 1140 |
| | GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT | 1200 |
| 40 | GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA | 1260 |
| | CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA | 1320 |
| 45 | TCAGTATCCG GAAATATTCG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA | 1380 |
| | GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG | 1440 |

| | | | | | | | |
|----|------------|------------|------------|------------|------------|------------|------|
| | CGCCAGCATG | GACAAATTCC | GTCAGGCGAT | GGGTATGATC | AAAAGCGCGG | TGGCGGGTGA | 1500 |
| | TACCGGCAAT | ACCAACCTGA | ACCTGCGTGG | CGCGGGCGGT | GCATCGCTGG | GTATCGATGC | 1560 |
| 5 | GGCTGTCTG | GGCGATAAAA | TAGCCAACAT | GTCGCTGGGT | AAGCTGGCCA | ACGCCTGATA | 1620 |
| | ATCTGTCTG | GCCTGATAAA | GCGGAAACGA | AAAAAGAGAC | GGGGAAGCCT | GTCTCTTTTC | 1680 |
| | TTATTATGCG | GTTTATGCGG | TTACCTGGAC | CGGTTAATCA | TCGTCATCGA | TCTGGTACAA | 1740 |
| 10 | ACGCACATTT | TCCCGTTCAT | TCGCGTCGTT | ACGCGCCACA | ATCGCGATGG | CATCTTCCTC | 1800 |
| | GTCGCTCAGA | TTGCGCGGCT | GATGGGGAAC | GCCGGGTGGA | ATATAGAGAA | ACTCGCCGGC | 1860 |
| 15 | CAGATGGAGA | CACGTCTGCG | ATAAATCTGT | GCCGTAACGT | GTTTCTATCC | GCCCCTTTAG | 1920 |
| | CAGATAGATT | GCGGTTTCGT | AATCAACATG | GTAATGCGGT | TCCGCCTGTG | CGCCGGCCGG | 1980 |
| | GATCACCACA | ATATTCATAG | AAAGCTGTCT | TGCACCTACC | GTATCGCGGG | AGATACCGAC | 2040 |
| 20 | AAAATAGGGC | AGTTTTTTCG | TGGTATCCGT | GGGGTGTTC | GGCCTGACAA | TCTTGAGTTG | 2100 |
| | GTTCGTCATC | ATCTTTCTCC | ATCTGGGCGA | CCTGATCGGT | T | | 2141 |

[illegible]

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| | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| | Leu | Gly | Ser | Lys | Gly | Gly | Asn | Asn | Thr | Thr | Ser | Thr | Thr | Asn | Ser | Pro | |
| | | | 115 | | | | | 120 | | | | | 125 | | | | |
| 5 | Leu | Asp | Gln | Ala | Leu | Gly | Ile | Asn | Ser | Thr | Ser | Gln | Asn | Asp | Asp | Ser | |
| | | 130 | | | | | 135 | | | | | 140 | | | | | |
| | Thr | Ser | Gly | Thr | Asp | Ser | Thr | Ser | Asp | Ser | Ser | Asp | Pro | Met | Gln | Gln | |
| | 145 | | | | | 150 | | | | | 155 | | | | 160 | | |
| 10 | Leu | Leu | Lys | Met | Phe | Ser | Glu | Ile | Met | Gln | Ser | Leu | Phe | Gly | Asp | Gly | |
| | | | | | 165 | | | | | 170 | | | | | 175 | | |
| | Gln | Asp | Gly | Thr | Gln | Gly | Ser | Ser | Ser | Gly | Gly | Lys | Gln | Pro | Thr | Glu | |
| | | | | 180 | | | | | 185 | | | | | 190 | | | |
| 15 | Gly | Glu | Gln | Asn | Ala | Tyr | Lys | Lys | Gly | Val | Thr | Asp | Ala | Leu | Ser | Gly | |
| | | | 195 | | | | | 200 | | | | | 205 | | | | |
| 20 | Leu | Met | Gly | Asn | Gly | Leu | Ser | Gln | Leu | Leu | Gly | Asn | Gly | Gly | Leu | Gly | |
| | | 210 | | | | | 215 | | | | | 220 | | | | | |
| | Gly | Gly | Gln | Gly | Gly | Asn | Ala | Gly | Thr | Gly | Leu | Asp | Gly | Ser | Ser | Leu | |
| | 225 | | | | | 230 | | | | | 235 | | | | | 240 | |
| 25 | Gly | Gly | Lys | Gly | Leu | Gln | Asn | Leu | Ser | Gly | Pro | Val | Asp | Tyr | Gln | Gln | |
| | | | | | 245 | | | | | 250 | | | | | 255 | | |
| | Leu | Gly | Asn | Ala | Val | Gly | Thr | Gly | Ile | Gly | Met | Lys | Ala | Gly | Ile | Gln | |
| | | | | 260 | | | | | 265 | | | | | 270 | | | |
| 30 | Ala | Leu | Asn | Asp | Ile | Gly | Thr | His | Arg | His | Ser | Ser | Thr | Arg | Ser | Phe | |
| | | | 275 | | | | | 280 | | | | | 285 | | | | |
| | Val | Asn | Lys | Gly | Asp | Arg | Ala | Met | Ala | Lys | Glu | Ile | Gly | Gln | Phe | Met | |
| 35 | | 290 | | | | | 295 | | | | | 300 | | | | | |
| | Asp | Gln | Tyr | Pro | Glu | Val | Phe | Gly | Lys | Pro | Gln | Tyr | Gln | Lys | Gly | Pro | |
| | 305 | | | | | 310 | | | | | 315 | | | | | 320 | |
| 40 | Gly | Gln | Glu | Val | Lys | Thr | Asp | Asp | Lys | Ser | Trp | Ala | Lys | Ala | Leu | Ser | |
| | | | | | 325 | | | | | 330 | | | | | 335 | | |
| | Lys | Pro | Asp | Asp | Asp | Gly | Met | Thr | Pro | Ala | Ser | Met | Glu | Gln | Phe | Asn | |
| | | | | 340 | | | | | 345 | | | | | 350 | | | |
| 45 | Lys | Ala | Lys | Gly | Met | Ile | Lys | Arg | Pro | Met | Ala | Gly | Asp | Thr | Gly | Asn | |
| | | | 355 | | | | | 360 | | | | | 365 | | | | |
| | Gly | Asn | Leu | Gln | Ala | Arg | Gly | Ala | Gly | Gly | Ser | Ser | Leu | Gly | Ile | Asp | |
| 50 | | 370 | | | | | 375 | | | | | 380 | | | | | |
| | Ala | Met | Met | Ala | Gly | Asp | Ala | Ile | Asn | Asn | Met | Ala | Leu | Gly | Lys | Leu | |
| | 385 | | | | | 390 | | | | | 395 | | | | | 400 | |
| 55 | Gly | Ala | Ala | | | | | | | | | | | | | | |

60 This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at

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least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

```

15  AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCATAA      60
    GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT      120
    ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG      180
20  GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATACCGT CAATCAGCTG      240
    GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG      300
25  GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA      360
    GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTTTCG TGAACACGCT GGGCTCGAAA      420
    GGCGGCAACA ATACCACTTC AACACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAAC      480
30  TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC      540
    CCGATGCAGC AGCTGCTGAA GATGTTGAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG      600
35  CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC      660
    GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG      720
    CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC      780
40  GGTTTCGTGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG      840
    TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTGAGGC GCTGAATGAT      900
45  ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG      960
    GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCCTGAGG TGTTTGGCAA GCCGCAGTAC      1020
    CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAAA AGCACTGAGC      1080
50  AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC      1140
    ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC      1200
55  GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA      1260
    CTTGGCAAGC TGGGCGCGGC TTAAGCTT      1288

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The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

5
Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
1 5 10 15
Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
10 20 25 30
Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
35 40 45
15 Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
50 55 60
Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
65 70 75 80
20 Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
85 90 95
Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
100 105 110
25 Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
115 120 125
30 Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
130 135 140
Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145 150 155 160
35 Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
165 170 175
Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
180 185 190
40 Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
195 200 205
Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
210 215 220
Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225 230 235 240
50 Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
245 250 255
Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
260 265 270
55 Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
275 280 285

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Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
 290 295 300
 5 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
 305 310 315 320
 Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
 325 330 335
 10 Asn Gln Ala Ala Ala
 340

This hypersensitive response elicitor polypeptide or
 15 protein has a molecular weight of 34-35 kDa. It is rich
 in glycine (about 13.5%) and lacks cysteine and tyrosine.
 Further information about the hypersensitive response
 elicitor derived from *Pseudomonas syringae* is found in
 He, S. Y., H. C. Huang, and A. Collmer, "*Pseudomonas*
 20 *syringae* pv. *syringae* Harpin_{Pss}: a Protein that is
 Secreted via the Hrp Pathway and Elicits the
 Hypersensitive Response in Plants," *Cell* 73:1255-1266
 (1993), which is hereby incorporated by reference. The
 DNA molecule encoding the hypersensitive response
 25 elicitor from *Pseudomonas syringae* has a nucleotide
 sequence corresponding to SEQ. ID. No. 6 as follows:

ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CTTGTCTCTG 60
 30 GTACGTCCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC 120
 GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA 180
 35 AAAGTGTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC 240
 ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG 300
 GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC 360
 40 AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC 420
 GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC 480
 AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC 540
 45 GAAACGGCTG CGTTCCGTTT GGCACGCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG 600
 AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC 660
 50 AACAACGCTG CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC 720
 GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA 780
 TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCAGCAGC CGGTACGTCC 840

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GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG 900
 GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT 960
 5 GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA 1020
 GCCTGA 1026

10 The hypersensitive response elicitor
 polypeptide or protein derived from *Pseudomonas*
solanacearum has an amino acid sequence corresponding to
 SEQ. ID. No. 7 as follows:

15 Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
 1 5 10 15
 Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
 20 20 25 30
 Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
 35 40 45
 25 Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
 50 55 60
 Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
 65 70 75 80
 30 Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
 85 90 95
 Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
 100 105 110
 35 Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
 115 120 125
 Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
 130 135 140
 40 Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
 145 150 155 160
 45 Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
 165 170 175
 Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
 180 185 190
 50 Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
 195 200 205
 55 Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
 210 215 220
 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
 225 230 235 240
 60

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Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
 245 250 255
 5 Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln
 260 265 270
 Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
 275 280 285
 10 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
 290 295 300
 Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
 305 310 315 320
 15 Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
 325 330 335
 20 Gln Ser Thr Ser Thr Gln Pro Met
 340

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ. ID. No. 8 as follows:

25 ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC 60
 AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC 120
 30 GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC 180
 GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC 240
 AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC 300
 35 GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA 360
 GACCTGGTGA AGCTGCTGAA GCGGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG 420
 40 GGCAACGGCG TGGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC 480
 GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC 540
 GCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT 600
 45 GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC 660
 GGCCCGCAGA ACGCAGGCGA TGTCACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC 720
 50 CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCGAG 780
 ATGATGCAGC AAGGCGGCCT CGGCGGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC 840
 GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT 900
 55 GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC 960
 GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG 1020
 60 ACGCAGCCGA TGTA 1035

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Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

```

15      Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
        1           5           10           15
      Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
                20           25

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20 This sequence is an amino terminal sequence having 26 residues only from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

25 The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 10 as follows:

```

35      Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
        1           5           10           15
      Leu Leu Ala Met
                20

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40 Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora*

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subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide is shown in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamoni*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from *Phytophthora*: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi *Phytophthora* Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path. 41:298-307 (1992), Baillreul et al., "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

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The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under which genes encoding an elicitor are expressed. Cell-free
5 preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

It is also possible to use fragments of the above hypersensitive response elicitor polypeptides or
10 proteins as well as fragments of full length elicitors from other pathogens, in the method of the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene
5 fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or a peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor
10 protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino
15 acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the
20 elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for

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increase and expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of a useful fragment is the popA1 fragment of the hypersensitive response elicitor polypeptide or protein from *Pseudomonas solanacearum*. See Arlat, M., F. Van Gijsegem, J.C. Huet, J.C. Pemollet, and C.A. Boucher, "PopA1, a Protein Which Induces a Hypersensitive-like Response in Specific Petunia Genotypes is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994), which is hereby incorporated by reference. As to *Erwinia amylovora*, a suitable fragment can be, for example, either or both the polypeptide extending between and including amino acids 1 and 98 of SEQ. ID. No. 3 and the polypeptide extending between and including amino acids 137 and 204 of SEQ. ID. No. 3.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide.

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The protein or polypeptide of the present invention is preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the hypersensitive response elicitor protein is separated by centrifugation. The supernatant fraction containing the polypeptide or protein of the present invention is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage

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and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

5 Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

 Suitable vectors include, but are not limited
10 to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning
15 Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes,"
20 Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard
25 cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

 A variety of host-vector systems may be
30 utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA;
35 microorganisms such as yeast containing yeast vectors;

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mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these
5 vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events
10 control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby
15 promotes mRNA synthesis. The DNA sequences of eucaryotic promoters differ from those of procaryotic promoters. Furthermore, eucaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further,
20 procaryotic promoters are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient
25 translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the
30 protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and

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Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of
5 expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For
10 instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*,
15 *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5* (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription
20 of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is
25 necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

30 Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein
35 synthesized, respectively. The DNA expression vector,

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which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon (ATG) to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The method of the present invention can be utilized to treat a wide variety of plants or their seeds to enhance growth. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: rose, *Saintpaulia*,

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petunia, pelargonium, poinsettia, chrysanthemum,
carnation, and zinnia.

The method of the present invention involving
application of the hypersensitive response elicitor
5 polypeptide or protein can be carried out through a
variety of procedures when all or part of the plant is
treated, including leaves, stems, roots, etc. This may
(but need not) involve infiltration of the hypersensitive
response elicitor polypeptide or protein into the plant.
10 Suitable application methods include topical application
(e.g., high or low pressure spraying), injection,
dusting, and leaf abrasion proximate to when elicitor
application takes place. When treating plant seeds, in
accordance with the application embodiment of the present
15 invention, the hypersensitive response elicitor protein
or polypeptide can be applied by topical application (low
or high pressure spraying), coating, immersion, dusting,
or injection. Other suitable application procedures can
be envisioned by those skilled in the art provided they
20 are able to effect contact of the hypersensitive response
elicitor polypeptide or protein with cells of the plant
or plant seed. Once treated with the hypersensitive
response elicitor of the present invention, the seeds can
be planted in natural or artificial soil and cultivated
25 using conventional procedures to produce plants. After
plants have been propagated from seeds treated in
accordance with the present invention, the plants may be
treated with one or more applications of the
hypersensitive response elicitor protein or polypeptide
30 to enhance growth in the plants. Such propagated plants
may, in turn, be useful in producing seeds or propagules
(e.g., cuttings) that produce plants capable of enhanced
growth.

The hypersensitive response elicitor
35 polypeptide or protein can be applied to plants or plant

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seeds in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor polypeptide or protein can be applied separately to plants with other materials
5 being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier.
10 Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 0.5 nM hypersensitive response elicitor polypeptide or protein.

Although not required, this composition may
15 contain additional additives including fertilizer, insecticide, fungicide, nematocide, herbicide, and mixtures thereof. Suitable fertilizers include $(\text{NH}_4)_2\text{NO}_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

20 Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor polypeptide or protein
25 can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and
30 transgenic seeds, a hypersensitive response elicitor polypeptide or protein need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein are produced
35 according to procedures well known in the art, such as by

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biolistics or *Agrobacterium* mediated transformation. Examples of suitable hypersensitive response elicitor polypeptides or proteins and the nucleic acid sequences for their encoding DNA are disclosed *supra*. Once
5 transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the hypersensitive response elicitor resulting in enhanced growth of the plant. Alternatively,
10 transgenic seeds are recovered from the transgenic plants. These seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions
15 effective to impart enhanced growth. While not wishing to be bound by theory, such growth enhancement may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used
20 in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor polypeptide or protein is applied. These other materials, including
25 hypersensitive response elicitors, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, dusting, and immersion. Similarly, after plants have been propagated from the transgenic
30 plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor to enhance plant growth. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.). The transgenic plants
35 of the present invention are useful in producing seeds or

propagules (e.g., cuttings) from which plants capable of enhanced growth would be produced.

EXAMPLES

5

**Example 1 - Effect of Treating Tomato Seeds with
Erwinia amylovora Hypersensitive Response
Elicitor on Germination Percentage**

10

Seeds of the Marglobe Tomato Variety were submerged in 40ml of *Erwinia amylovora* hypersensitive response elicitor solution ("harpin"). Harpin was prepared by growing *E. coli* strain DH5 containing the plasmid pCPP2139 (see Figure 1), lysing the cells by sonication, heat treating by holding in boiling water for 15 5 minutes before centrifuging to remove cellular debris, and precipitating proteins and other heat-labile components. The resulting preparation ("CFEP") was diluted serially. These dilutions (1:40, 1:80, 1:160, 20 1:320 and 1:640) contained 20, 10, 5, 2.5, and 1.25 $\mu\text{gm/ml}$, respectively, of harpin based on Western Blot assay. Seeds were soaked in harpin or buffer in beakers on day 0 for 24 hours at 28°C in a growth chamber. After soaking, the seeds were sown in germination pots with 25 artificial soil on day 1. This procedure was carried out on 100 seeds per treatment.

Treatments:

30

1. Seeds in harpin (1:40) (20 $\mu\text{gm/ml}$).
2. Seeds in harpin (1:80) (10 $\mu\text{gm/ml}$).
3. Seeds in harpin (1:160) (5 $\mu\text{gm/ml}$).
4. Seeds in harpin (1:320) (2.5 $\mu\text{gm/ml}$).
5. Seeds in harpin (1:640) (1.25 $\mu\text{gm/ml}$).
6. Seeds in buffer (5mM KPO_4 , pH 6.8).

35

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Table 1 - Number of Seedlings After Seed Treatment

| Treatment | Number of seeds germinated | | | | |
|--|----------------------------|-------|-------|-------|-------|
| | Day 0 | Day 1 | Day 5 | Day 7 | Day 9 |
| 5 Harpin seed soak (20 μ gm/ml) | sowing | | 43 | 57 | 59 |
| Harpin seed soak (10 μ gm/ml) | sowing | | 43 | 52 | 52 |
| Harpin seed soak (5 μ gm/ml) | sowing | | 40 | 47 | 51 |
| Harpin seed soak (2.5 μ gm/ml) | sowing | | 43 | 56 | 58 |
| 10 Harpin seed soak (1.25 μ gm/ml) | sowing | | 38 | 53 | 57 |
| Buffer seed soak | sowing | | 27 | 37 | 40 |

As shown in Table 1, the treatment of tomato seeds with *Erwinia amylovora* hypersensitive response elicitor reduced the time needed for germination and greatly increased the percentage of germination.

Example 2 - Effect of Treating Tomato Seeds with *Erwinia amylovora* Hypersensitive Response Elicitor on Tomato Plant Height

Seeds of the Marglobe Tomato Variety were submerged in *Erwinia amylovora* harpin (1:15, 1:30, 1:60, and 1:120) or buffer in beakers on day 0 for 24 hours at 28°C in a growth chamber. After soaking, the seeds were sown in germination pots with artificial soil on day 1.

Ten uniform appearing plants per treatment were chosen randomly and measured. The seedlings were measured by ruler from the surface of soil to the top of plant.

Treatments:

1. Harpin (1:15) (52 μ gm/ml).
2. Harpin (1:30) (26 μ gm/ml).
3. Harpin (1:60) (13 μ gm/ml).
4. Harpin (1:120) (6.5 μ gm/ml).
5. Buffer (5mM KPO₄, pH 6.8).

Table 2 - Seedling Height (cm) 15 Days After Seed Treatment.

| Treat | Plants | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
|-----------------|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| 52 μ gm/ml | 10 | 5.6 | 5.8 | 5.8 | 5.6 | 6.0 | 6.0 | 5.8 | 5.4 | 5.8 | 5.6 | 5.7 |
| 26 μ gm/ml | 10 | 6.8 | 7.2 | 6.6 | 7.0 | 6.8 | 6.8 | 7.0 | 7.4 | 7.2 | 7.0 | 7.0 |
| 13 μ gm/ml | 10 | 5.8 | 5.6 | 6.0 | 5.6 | 5.8 | 5.8 | 5.6 | 5.8 | 6.0 | 5.6 | 5.9 |
| 6.5 μ gm/ml | 10 | 5.4 | 5.2 | 5.6 | 5.4 | 5.2 | 5.4 | 5.6 | 5.6 | 5.4 | 5.2 | 5.4 |
| Buffer | 10 | 5.6 | 5.4 | 5.2 | 5.2 | 5.4 | 5.2 | 5.0 | 5.2 | 5.4 | 5.6 | 5.3 |

Table 3 - Seedling Height (cm) 21 Days After Seed Treatment.

| Treat | Plants | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
|-----------------|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| 52 μ gm/ml | 10 | 7.6 | 7.8 | 7.6 | 7.6 | 7.8 | 7.8 | 7.8 | 7.4 | 7.6 | 7.6 | 7.7 |
| 26 μ gm/ml | 10 | 8.2 | 8.2 | 8.0 | 9.0 | 8.4 | 8.6 | 8.6 | 9.0 | 9.2 | 9.0 | 8.6 |
| 13 μ gm/ml | 10 | 6.8 | 6.6 | 6.8 | 6.8 | 6.8 | 6.8 | 6.6 | 7.2 | 7.0 | 7.2 | 6.9 |
| 6.5 μ gm/ml | 10 | 6.8 | 6.6 | 6.6 | 6.4 | 6.8 | 6.6 | 6.8 | 6.6 | 6.6 | 6.8 | 6.7 |
| Buffer | 10 | 6.6 | 6.4 | 6.2 | 6.6 | 6.4 | 6.6 | 6.8 | 6.4 | 6.4 | 6.6 | 6.5 |

Table 4 - Seedling Height (cm) 27 Days After Seed Treatment.

| Treat | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
|-----------------|------|------|------|------|------|------|------|------|------|------|------|
| 52 μ gm/ml | 10.2 | 10.6 | 10.4 | 10.6 | 10.4 | 10.6 | 10.8 | 10.4 | 10.8 | 10.6 | 10.5 |
| 26 μ gm/ml | 11.6 | 11.4 | 11.6 | 11.8 | 11.8 | 11.8 | 11.6 | 11.4 | 11.6 | 11.4 | 11.6 |
| 13 μ gm/ml | 9.8 | 9.6 | 9.8 | 9.6 | 9.8 | 9.8 | 9.6 | 9.4 | 9.6 | 9.8 | 9.7 |
| 6.5 μ gm/ml | 9.4 | 9.4 | 9.6 | 9.4 | 9.6 | 9.4 | 9.6 | 9.6 | 9.4 | 9.2 | 9.5 |
| Buffer | 9.6 | 10.2 | 10.0 | 9.8 | 10.0 | 10.2 | 10.0 | 10.2 | 10.4 | 9.6 | 10.0 |

Table 5 - Summary--Mean Height of Tomato Plants after Treatment.

| 5 | Treatment | Mean height of tomato plants (cm) | | | | |
|----|--------------------------|-----------------------------------|--------|--------|--------|--|
| | Day 0 | Day 1 | Day 15 | Day 21 | Day 27 | |
| 10 | Harpin seed soak (1:15) | sowing | 5.7 | 7.7 | 10.5 | |
| | Harpin seed soak (1:30) | sowing | 7.0 | 8.6 | 11.6 | |
| | Harpin seed soak (1:60) | sowing | 5.9 | 6.9 | 9.7 | |
| | Harpin seed soak (1:120) | sowing | 5.4 | 6.7 | 9.5 | |
| | Buffer seed soak | sowing | 5.3 | 6.5 | 10.0 | |

As shown in Tables 2-5, the treatment of tomato seeds with *Erwinia amylovora* hypersensitive response elicitor increased plant growth. A 1:30 dilution had the greatest effect -- a 16% increase in seedling height.

Example 3 - Effect of Treating Tomato Plants with *Erwinia amylovora* Hypersensitive Response Elicitor on Tomato Plant Height

When Marglobe tomato plants were 4 weeks old, they were sprayed with 6 ml/plant of *Erwinia amylovora* harpin solution containing 13 $\mu\text{gm/ml}$ (1:60) or 8.7 $\mu\text{gm/ml}$ (1:90) of harpin or buffer (5mM KPO_4) in a growth chamber at 28°C. The heights of tomato plants were measured 2 weeks after spraying harpin (6-week-old tomato plants) and 2 weeks plus 5 days after spraying. Ten uniform appearing plants per treatment were chosen randomly and measured. The seedlings were measured by ruler from the surface of soil to the top of plant.

Treatments:

1. Harpin (1:60) (13 $\mu\text{gm/ml}$).
2. Harpin (1:90) (8.7 $\mu\text{gm/ml}$).
3. Buffer (5mM KPO_4 , pH 6.8).

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Table 6 - Mean Height of Tomato Plants after Treatment With Harpin.

| 5 | Operation and Treatment | | | Mean height (cm) of tomato plants | |
|----|-------------------------|------------|----------------------------------|--------------------------------------|--------|
| | Day 0 | Day 14 | Day 28 | Day 42 | Day 47 |
| 10 | sowing | transplant | harpin 1:60 (13 μ gm/ml) | 35.5 | 36.0 |
| | sowing | transplant | harpin 1:90 (8.7 μ gm/ml) | 35.7 | 36.5 |
| 15 | sowing | transplant | buffer | 32.5 | 33.0 |

As shown in Table 6, spraying tomato seedlings with *Erwinia amylovora* hypersensitive response elicitor can increase growth of tomato plants. Similar increases in growth were noted for the two doses of the hypersensitive response elicitor tested compared with the buffer-treated control.

Example 4 - Effect of Treating Tomato Seeds with *Erwinia amylovora* Hypersensitive Response Elicitor on Tomato Plant Height

Marglobe tomato seeds were submerged in *Erwinia amylovora* hypersensitive response elicitor solution ("harpin") (1:40, 1:80, 1:160, 1:320, and 1:640) or buffer in beakers on day 0 for 24 hours at 28°C in the growth chamber. After soaking seeds in harpin or buffer, they were sown in germination pots with artificial soil on day 1. Ten uniform appearing plants per treatment were chosen randomly and measured. The seedlings were measured by ruler from the surface of soil to the top of plant.

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Treatments:

1. Harpin (1:40) (20 $\mu\text{gm/ml}$).
2. Harpin (1:80) (10 $\mu\text{gm/ml}$).
3. Harpin (1:160) (5 $\mu\text{gm/ml}$).
4. Harpin (1:320) (2.5 $\mu\text{gm/ml}$).
5. Harpin (1:640) (1.25 $\mu\text{gm/ml}$).
6. Buffer (5mM KPO_4 , pH 6.8).

Table 7 - Seedling Height (cm) 12 Days After Seed Treatment.

| Treat | Plants | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
|------------------|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| 20 μ gm/ml | 10 | 6.5 | 6.8 | 6.8 | 6.5 | 6.4 | 6.4 | 6.8 | 6.4 | 6.8 | 6.6 | 6.6 |
| 10 μ gm/ml | 10 | 6.8 | 6.2 | 6.6 | 6.4 | 6.8 | 6.8 | 6.6 | 6.4 | 6.8 | 6.4 | 6.6 |
| 5 μ gm/ml | 10 | 6.2 | 6.6 | 6.0 | 6.6 | 6.4 | 6.2 | 6.6 | 6.2 | 6.0 | 6.6 | 6.3 |
| 2.5 μ gm/ml | 10 | 6.4 | 6.2 | 6.6 | 6.0 | 6.2 | 6.4 | 6.0 | 6.0 | 6.2 | 6.2 | 6.2 |
| 1.25 μ gm/ml | 10 | 6.2 | 6.2 | 6.0 | 6.4 | 6.0 | 6.0 | 6.4 | 6.2 | 6.4 | 6.2 | 6.2 |
| Buffer | 10 | 5.8 | 6.0 | 6.2 | 6.2 | 5.8 | 5.8 | 6.0 | 6.2 | 6.0 | 6.0 | 6.0 |

Table 8 - Seedling Height (cm) 14 Days After Seed Treatment.

| Treat | Plants | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
|------------------|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| 20 μ gm/ml | 10 | 7.8 | 7.8 | 8.2 | 8.0 | 8.2 | 8.4 | 7.8 | 8.4 | 7.6 | 7.8 | 8.0 |
| 10 μ gm/ml | 10 | 8.6 | 8.8 | 8.4 | 9.2 | 8.4 | 8.6 | 7.8 | 7.8 | 8.4 | 8.4 | 8.4 |
| 5 μ gm/ml | 10 | 9.8 | 9.2 | 9.8 | 9.6 | 9.2 | 9.4 | 8.6 | 9.2 | 9.0 | 8.6 | 9.2 |
| 2.5 μ gm/ml | 10 | 8.8 | 8.6 | 8.6 | 8.4 | 7.8 | 8.6 | 8.4 | 9.0 | 8.0 | 7.8 | 8.4 |
| 1.25 μ gm/ml | 10 | 8.4 | 7.8 | 8.4 | 8.0 | 8.6 | 8.4 | 8.0 | 8.2 | 8.4 | 8.2 | 8.2 |
| Buffer | 10 | 7.2 | 8.2 | 7.4 | 7.6 | 7.8 | 7.6 | 7.8 | 7.4 | 7.8 | 7.6 | 7.6 |

Table 9 - Seedling Height (cm) 17 Days After Seed Treatment.

| Treat | Plants | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
|------------------|--------|------|------|------|------|------|------|------|------|------|------|------|
| 20 μ gm/ml | 10 | 11.2 | 11.6 | 11.4 | 11.6 | 11.4 | 11.2 | 11.8 | 11.4 | 11.8 | 11.6 | 11.5 |
| 10 μ gm/ml | 10 | 13.4 | 13.4 | 13.8 | 13.2 | 13.4 | 12.6 | 12.4 | 13.4 | 13.2 | 13.4 | 13.2 |
| 5 μ gm/ml | 10 | 13.6 | 12.8 | 13.6 | 13.2 | 14.2 | 13.8 | 12.6 | 13.4 | 13.8 | 13.6 | 13.5 |
| 2.5 μ gm/ml | 10 | 11.6 | 12.4 | 12.4 | 11.8 | 11.6 | 12.2 | 12.6 | 11.8 | 12.0 | 11.6 | 12.0 |
| 1.25 μ gm/ml | 10 | 12.8 | 12.6 | 12.0 | 12.4 | 11.6 | 11.8 | 12.2 | 11.4 | 11.2 | 11.4 | 11.9 |
| Buffer | 10 | 10.0 | 10.4 | 10.6 | 10.6 | 10.4 | 10.4 | 10.8 | 10.2 | 10.4 | 10.0 | 10.4 |

Table 10 -Summary - Mean Height of Tomato Plants After Treatment

| Operation and Treatment | Mean height of tomato plants (cm) | | | | | | |
|-------------------------------------|--------------------------------------|--------|--------|--------|--------|--|--|
| | Day 0 | Day 1 | Day 12 | Day 14 | Day 17 | | |
| Harpin seed soak (20 μ gm/ml) | sowing | sowing | 6.6 | 8.0 | 11.5 | | |
| Harpin seed soak (10 μ gm/ml) | sowing | sowing | 6.6 | 8.4 | 13.2 | | |
| Harpin seed soak (5 μ gm/ml) | sowing | sowing | 6.3 | 9.2 | 13.5 | | |
| Harpin seed soak (2.5 μ gm/ml) | sowing | sowing | 6.2 | 8.4 | 12.0 | | |
| Harpin seed soak (1.25 μ gm/ml) | sowing | sowing | 6.2 | 8.2 | 11.9 | | |
| Buffer seed soak | sowing | sowing | 6.0 | 7.6 | 10.4 | | |

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As shown in Tables 7-10, the treatment of tomato seeds with *Erwinia amylovora* hypersensitive response elicitor can increase growth of tomato plants. A 1:160 dilution (5 $\mu\text{g}/\text{ml}$ harpin) had the greatest effect -- seedling
5 height was increased more than 20% over the buffer treated plants.

10 **Example 5 - Effect of Treating Tomato Seeds with *Erwinia amylovora* Hypersensitive Response Elicitor on Seed Germination Percentage**

Marglobe tomato seeds were submerged in 40ml of *Erwinia amylovora* hypersensitive response elicitor ("harpin") solution (dilutions of CFEP from *E. coli* DH5
15 (pCPP2139) of 1:50 or 1:100 which contained, respectively, 8 $\mu\text{g}/\text{ml}$ and 4 $\mu\text{g}/\text{ml}$ of hypersensitive response elicitor) and buffer in beakers on day 0 for 24 hours at 28°C in a growth chamber. After soaking, the seeds were sown in germination pots with artificial soil
20 on day 1. This treatment was carried out on 20 seeds per pot and 4 pots per treatment.

Treatments:

- 25 1. Harpin (8 $\mu\text{g}/\text{ml}$).
2. Harpin (8 $\mu\text{g}/\text{ml}$).
3. Harpin (8 $\mu\text{g}/\text{ml}$).
4. Harpin (8 $\mu\text{g}/\text{ml}$).
5. Harpin (4 $\mu\text{g}/\text{ml}$).
6. Harpin (4 $\mu\text{g}/\text{ml}$).
- 30 7. Harpin (4 $\mu\text{g}/\text{ml}$).
8. Harpin (4 $\mu\text{g}/\text{ml}$).
9. Buffer (5mM KPO_4 , pH 6.8).
10. Buffer (5mM KPO_4 , pH 6.8).
11. Buffer (5mM KPO_4 , pH 6.8).
- 35 12. Buffer (5mM KPO_4 , pH 6.8).

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Table 11 - Number of Seedlings After Seed Treatment With Harpin

| 5 | Operation and Treatment | | | | Number of seeds germinated (out of a total of 20) | | | |
|----|-------------------------|--------|-------|------|--|------|--------|------|
| | Day 0 | Day 1 | Day 5 | Mean | Day 42 | Mean | Day 47 | Mean |
| 10 | Harpin (8 µgm/ml) | sowing | 11 | | 15 | | 19 | |
| | Harpin (8 µgm/ml) | sowing | 13 | | 17 | | 20 | |
| | Harpin (8 µgm/ml) | sowing | 10 | | 13 | | 16 | |
| | Harpin (8 µgm/ml) | sowing | 9 | 10.8 | 15 | 15.0 | 16 | 17.8 |
| 15 | Harpin (4 µgm/ml) | sowing | 11 | | 17 | | 17 | |
| | Harpin (4 µgm/ml) | sowing | 15 | | 17 | | 18 | |
| | Harpin (4 µgm/ml) | sowing | 9 | | 12 | | 14 | |
| | Harpin (4 µgm/ml) | sowing | 9 | 11.0 | 14 | 15.0 | 16 | 16.3 |
| 20 | Buffer | sowing | 11 | | 11 | | 14 | |
| | Buffer | sowing | 9 | | 14 | | 15 | |
| | Buffer | sowing | 10 | | 14 | | 14 | |
| | Buffer | sowing | 10 | 10.0 | 12 | 12.8 | 14 | 14.3 |

25

As shown in Table 11, treatment of tomato seeds with *Erwinia amylovora* hypersensitive response elicitor can increase germination rate and level of tomato seeds. The higher dose used appeared to be more effective than buffer at the end of the experiment.

5

Example 6 - Effect on Plant Growth of Treating Tomato Seeds with Proteins Prepared from *E. coli* Containing a Hypersensitive Response Elicitor Encoding Construct, pCPP2139, or Plasmid Vector pCPP50

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Marglobe tomato seeds were submerged in *Erwinia amylovora* hypersensitive response elicitor ("harpin") (from *E. coli* DH5α(pCPP2139) (Figure 1) or vector preparation (from DH5α(pCPP50) (Figure 2) with added BSA protein as control. The control vector preparation contained, per ml, 33.6 µl of BSA (10 mg/ml) to provide about the same amount of protein as contained in the

15

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pCPP2139 preparation due to harpin. Dilutions of 1:50 (8.0 $\mu\text{g/ml}$), 1:100 (4.0 $\mu\text{g/ml}$), and 1:200 (2.0 $\mu\text{g/ml}$) were prepared in beakers on day 1, and seed was submerged for 24 hours at 28°C in a controlled environment chamber.

- 5 After soaking, seeds were sown in germination pots with artificial soil on day 2. Ten uniform appearing plants per treatment were chosen randomly and measured at three times after transplanting. The seedlings were measured by ruler from the surface of soil to the top of plant.

10

Treatments:

- | | | | |
|----|-----------------|-------|-------------------------|
| | 1. Harpin | 1:50 | (8.0 $\mu\text{g/ml}$) |
| | 2. Harpin | 1:100 | (4.0 $\mu\text{g/ml}$) |
| | 3. Harpin | 1:200 | (2.0 $\mu\text{g/ml}$) |
| 15 | 4. Vector + BSA | 1:50 | (0 harpin) |
| | 5. Vector + BSA | 1:100 | (0 harpin) |
| | 6. Vector + BSA | 1:200 | (0 harpin) |

Table 12 - Seedling Height (cm) 18 Days After Seed Treatment

| Treat | Harpin | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
|--------|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| H1:50 | 8.0 | 3.6 | 5.0 | 4.8 | 5.0 | 4.2 | 5.2 | 5.8 | 4.6 | 4.0 | 4.8 | 4.7 |
| H1:100 | 4.0 | 4.6 | 5.8 | 6.2 | 6.0 | 5.6 | 6.8 | 6.0 | 4.8 | 5.6 | 6.2 | 5.8 |
| H1:200 | 2.0 | 4.0 | 5.8 | 5.8 | 4.6 | 5.4 | 5.0 | 5.8 | 4.6 | 4.6 | 5.8 | 5.1 |
| V1:50 | 0 | 3.8 | 5.0 | 4.6 | 5.4 | 5.6 | 4.6 | 5.0 | 5.2 | 4.6 | 4.8 | 4.9 |
| V1:100 | 0 | 4.4 | 5.2 | 4.6 | 4.4 | 5.4 | 4.8 | 5.0 | 4.6 | 4.4 | 5.2 | 4.8 |
| V1:200 | 0 | 4.2 | 4.8 | 5.4 | 4.6 | 5.0 | 4.8 | 4.8 | 5.4 | 4.6 | 5.0 | 4.9 |

Table 13 - Seedling Height (cm) 22 Days After Seed Treatment.

| Treat | Harpin | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
|--------|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| H1:50 | 8.0 | 4.2 | 5.6 | 5.2 | 6.0 | 4.8 | 5.4 | 5.0 | 5.2 | 5.4 | 5.0 | 5.2 |
| H1:100 | 4.0 | 7.6 | 6.8 | 7.0 | 7.2 | 6.8 | 7.4 | 7.6 | 7.0 | 6.8 | 7.4 | 7.2 |
| H1:200 | 2.0 | 7.0 | 6.6 | 6.8 | 7.2 | 7.4 | 6.8 | 7.0 | 7.2 | 6.8 | 7.2 | 7.0 |
| V1:50 | 0 | 5.6 | 5.8 | 6.2 | 6.4 | 5.6 | 5.2 | 5.6 | 5.8 | 6.0 | 5.8 | 5.8 |
| V1:100 | 0 | 5.4 | 6.0 | 5.8 | 6.2 | 5.8 | 5.6 | 5.4 | 5.2 | 6.0 | 5.6 | 5.7 |
| V1:200 | 0 | 5.2 | 6.2 | 5.8 | 5.4 | 6.2 | 6.0 | 5.6 | 6.4 | 5.8 | 6.0 | 5.9 |

Table 14 - Seedling Height (cm) 26 Days After Seed Treatment.

| Treat. | Harpin | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
|--------|--------|------|------|------|------|------|------|------|------|------|------|------|
| H1:50 | 8.0 | 7.6 | 8.4 | 8.8 | 6.8 | 9.6 | 8.2 | 7.4 | 9.8 | 9.2 | 9.0 | 8.5 |
| H1:100 | 4.0 | 12.0 | 11.4 | 11.2 | 11.0 | 10.8 | 12.0 | 11.2 | 11.6 | 10.4 | 10.2 | 11.2 |
| H1:200 | 2.0 | 10.6 | 11.2 | 11.6 | 10.2 | 11.0 | 10.8 | 10.0 | 11.8 | 10.2 | 10.6 | 10.8 |
| V1:50 | 0 | 9.0 | 9.4 | 8.8 | 8.4 | 9.6 | 9.2 | 9.2 | 8.6 | 8.0 | 9.4 | 9.2 |
| V1:100 | 0 | 9.2 | 10.0 | 9.8 | 9.6 | 8.4 | 9.4 | 9.6 | 9.8 | 8.0 | 9.6 | 9.3 |
| V1:200 | 0 | 8.8 | 9.6 | 8.2 | 9.2 | 8.4 | 8.0 | 9.8 | 9.0 | 9.4 | 9.2 | 9.0 |

Table 15 - Mean Height of Tomato Plants After Treatment

| Operation and Treatment | | Mean height of tomato plants (cm) | | | |
|----------------------------------|--------|-----------------------------------|--------|--------|--|
| Day 1 | Day 2 | Day 18 | Day 22 | Day 26 | |
| Harpin (1:50) (8.0 μ gm/ml) | sowing | 4.7 | 5.2 | 8.5 | |
| Harpin (1:100) (4.0 μ gm/ml) | sowing | 5.8 | 7.2 | 11.2 | |
| Harpin (1:200) (2.0 μ gm/ml) | sowing | 5.1 | 7.0 | 10.8 | |
| Vector + BSA (1:50) (0) | sowing | 4.9 | 5.8 | 9.2 | |
| Vector + BSA (1:100) (0) | sowing | 4.8 | 5.7 | 9.3 | |
| Vector + BSA (1:200) (0) | sowing | 4.9 | 5.9 | 9.0 | |

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As shown in Tables 12-15, treatment with *E. coli* containing the gene encoding the *Erwinia amylovora* hypersensitive response elicitor can increase growth of tomato plants. The 1:100 dilution (4.0 $\mu\text{g/ml}$) had the greatest effect, while higher and lower concentrations had less effect. Mean seedling height for treatment with 4.0 $\mu\text{g/ml}$ of harpin was increased about 20% relative to vector control preparation, which contained a similar amount of non-harpin protein. Components of the lysed cell preparation from the strain *E. coli* DH5 α (pCPP50), which harbors the vector of the *hrpN* gene in *E. coli* strain DH5 α (pCPP2139), do not have the same growth-promoting effect as the harpin-containing preparation, even given that it is supplemented with BSA protein to the same extent as the DH5 α (pCPP2139) preparation, which contains large amounts of harpin protein.

Example 7 - Effect on Tomato Plant Growth of Treating Tomato Seeds with Proteins Prepared from *E. coli* Containing a Hypersensitive Response Elicitor Encoding Construct, pCPP2139, or its Plasmid Vector pCPP50

Marglobe tomato seeds were submerged in *Erwinia amylovora* hypersensitive response elicitor solution ("harpin") (from the harpin encoding plasmid pCPP2139 vector) and from pCPP50 vector-containing solution at dilutions of 1:25, 1:50, and 1:100 in beakers on day 1 for 24 hours at 28°C in a growth chamber. After soaking seeds, they were sown in germination pots with artificial soil on day 2. Ten uniform appearing plants per treatment were chosen randomly and measured. The seedlings were measured by ruler from the surface of soil to the top of plant.

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Treatments:

1. Harpin 16 $\mu\text{gm/ml}$
2. Harpin 8 $\mu\text{gm/ml}$
3. Harpin 4 $\mu\text{gm/ml}$
4. Vector 16 $\mu\text{gm/ml}$
5. Vector 8 $\mu\text{gm/ml}$
6. Vector 4 $\mu\text{gm/ml}$

Table 16 - Seedling Height (cm) 11 Days After Seed Treatment

| Treat. | Harpin | Plants | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
|--------|----------------|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| H1:25 | 16 μ gm/ml | 10 | 5.0 | 5.2 | 4.8 | 4.6 | 4.4 | 4.6 | 3.8 | 4.2 | 3.8 | 4.2 | 4.5 |
| H1:50 | 8 μ gm/ml | 10 | 5.6 | 5.4 | 6.0 | 5.8 | 4.8 | 6.8 | 5.8 | 5.0 | 5.2 | 4.8 | 5.5 |
| H1:100 | 4 μ gm/ml | 10 | 5.2 | 5.6 | 5.0 | 5.0 | 5.0 | 4.8 | 5.0 | 5.6 | 4.8 | 5.2 | 5.1 |
| V1:25 | 0 | 10 | 4.4 | 4.4 | 4.8 | 4.6 | 4.8 | 4.6 | 4.0 | 4.8 | 4.4 | 4.6 | 4.5 |
| V1:50 | 0 | 10 | 4.8 | 4.4 | 4.6 | 4.0 | 4.4 | 4.2 | 4.6 | 4.0 | 4.4 | 4.2 | 4.4 |
| V1:100 | 0 | 10 | 4.6 | 4.2 | 4.8 | 4.4 | 4.4 | 4.0 | 4.2 | 4.0 | 4.4 | 4.0 | 4.3 |

Table 17 - Seedling Height (cm) 14 Days After Seed Treatment

| Treat. | Harpin | Plants | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
|--------|----------------|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| H1:25 | 16 μ gm/ml | 10 | 7.6 | 7.6 | 7.2 | 7.4 | 7.8 | 7.8 | 7.6 | 7.0 | 7.4 | 7.0 | 7.4 |
| H1:50 | 8 μ gm/ml | 10 | 8.5 | 8.2 | 8.4 | 7.6 | 7.8 | 8.4 | 8.6 | 9.0 | 7.6 | 8.2 | 8.2 |
| H1:100 | 4 μ gm/ml | 10 | 7.2 | 8.4 | 8.2 | 7.4 | 8.0 | 7.6 | 7.6 | 8.0 | 8.6 | 7.6 | 7.9 |
| V1:25 | 0 | 10 | 6.8 | 6.4 | 7.8 | 6.6 | 6.6 | 6.8 | 7.4 | 6.0 | 6.4 | 6.4 | 6.7 |
| V1:50 | 0 | 10 | 6.6 | 5.8 | 6.4 | 7.6 | 7.4 | 7.2 | 6.8 | 6.6 | 6.4 | 5.8 | 6.7 |
| V1:100 | 0 | 10 | 6.2 | 6.0 | 6.8 | 6.6 | 6.4 | 5.8 | 6.6 | 7.0 | 5.8 | 6.4 | 6.4 |

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Table 18 - Mean Height of Tomato Plants After Treatment.

| 5 | Operation and Treatment | Mean height of tomato plants (cm) | | | |
|----|-----------------------------------|-----------------------------------|-------|--------|--------|
| | | Day 1 | Day 2 | Day 11 | Day 14 |
| | Harpin seed soak (16 μ gm/ml) | sowing | 4.5 | 7.4 | |
| 10 | Harpin seed soak (8 μ gm/ml) | sowing | 5.5 | 8.2 | |
| | Harpin seed soak (4 μ gm/ml) | sowing | 5.1 | 7.9 | |
| | Vector seed soak (16 μ gm/ml) | sowing | 4.5 | 6.7 | |
| | Vector seed soak (8 μ gm/ml) | sowing | 4.4 | 6.7 | |
| | Vector seed soak (4 μ gm/ml) | sowing | 4.3 | 6.4 | |
| 15 | ----- | | | | |

As shown in Tables 16-18, treatment with *Erwinia amylovora* hypersensitive response elicitor can increase growth of tomato plants. A 1:50 dilution (8 μ g/ml hypersensitive response elicitor) had the greatest effect with seedling height being increased by about 20% over the control.

Example 8 - Effect of Cell-Free *Erwinia amylovora* Hypersensitive Response Elicitor on Growth of Potato

Three-week-old potato plants, variety Norchip, were grown from tuber pieces in individual containers. The foliage of each plant was sprayed with a solution containing *Erwinia amylovora* hypersensitive response elicitor ("harpin"), or a control solution containing proteins of *E. coli* and those of the vector pCPP50 ("vector"), diluted 1:50, 1:100, and 1:200. On day 20, 12 uniform appearing plants were chosen randomly for each of the following treatments. One plant from each treatment was maintained at 16°C, in a growth chamber, while two plants from each treatment were maintained on a greenhouse bench at 18-25°C. Twenty-five days after treatment, the shoots (stems) on all plants were measured individually.

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Treatments:

| | |
|-----------------|----------------|
| 1. Harpin 1:50 | 16 μ gm/ml |
| 2. Harpin 1:100 | 8 μ gm/ml |
| 3. Harpin 1:200 | 4 μ gm/ml |
| 4. Vector 1:50 | 0 harpin |
| 5. Vector 1:100 | 0 harpin |
| 6. Vector 1:200 | 0 harpin |

Table 19 - Length of Potato Stems of Plants at 16°C

| Treatment on day 20 | Length of potato stems (cm) stem on day 45 | | | | | |
|---------------------|--|--------|------------|------------|-----------------|------------|
| | stem 1 | stem 2 | stem 3 | stem 4 | stem 5 | Plant Mean |
| Harpin 1:50 | 43.0 | 39.5 | 42.5 | 34.0 | 38.0 | 39.4 |
| Harpin 1:100 | 42.0 | 38.5 | (2 branch) | | 39.5 | 40.3 |
| Harpin 1:200 | 35.5 | 30.5 | 31.5 | (3 branch) | | 32.5 |
| Vector 1:50 | 34.0 | 32.0 | 31.5 | 28.0 | 27.5 (5 branch) | 30.6 |
| Vector 1:100 | 30.0 | 33.5 | 33.0 | 30.0 | 28.0 | 31.3 |
| Vector 1:200 | 33.5 | 31.5 | 32.5 | (3 branch) | 33.0 | 32.5 |

Table 20 - Length of Potato Stems of Plants on a Greenhouse Bench

| Treatment on day 20 | Length of potato stems (cm) on day 45 | | | | | | Treat. Mean |
|---------------------|---------------------------------------|--------|------------|------------|------------|------------|----------------|
| | stem 1 | stem 2 | stem 3 | stem 4 | stem 5 | Plant | |
| Harpin 1:50 | 65.5 | 58.5 | 57.5 | 62.5 | 68.5 | (5 branch) | 62.5 |
| Harpin 1:100 | 62.5 | 67.0 | 65.0 | 69.0 | (4 branch) | | 65.9 |
| Harpin 1:200 | 70.5 | 73.5 | 74.0 | 80.5 | (4 branch) | | 74.6 |
| Vector 1:50 | 83.0 | 80.5 | 76.5 | 76.0 | 81.5 | (5 branch) | 79.5 |
| Vector 1:100 | 56.5 | 59.5 | 50.5 | 53.0 | 55.5 | 48.0 | 53.9 |
| Vector 1:200 | 57.0 | 59.5 | 69.5 | (3 branch) | | | 62.0 |
| Vector 1:50 | 53.0 | 62.0 | 59.5 | 62.5 | (4 branch) | | 59.3 |
| Vector 1:100 | 52.0 | 46.0 | 61.5 | 56.5 | 61.5 | 57.0 | 55.8 |
| Vector 1:200 | 62.0 | 51.5 | 66.0 | 67.5 | 62.0 | 63.0 | 62.0 |
| Vector 1:50 | 61.5 | 62.5 | 59.0 | 65.5 | 63.0 | 63.5 | 62.5 |
| Vector 1:100 | 62.0 | 66.0 | (2 branch) | | | | 64.0 |
| Vector 1:200 | 61.0 | 60.0 | 63.5 | (3 branch) | | | 61.5 |
| Vector 1:200 | | | | | | | 62.8 |

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As shown in Tables 19 and 20, treatment of potato plants with *Erwinia amylovora* hypersensitive response elicitor enhanced shoot (stem) growth. Thus, overall growth, as judged by both the number and mean
5 lengths of stems, were greater in the harpin-treated plants in both the greenhouse and growth chamber-grown plants. The potato plants treated with the medium dose of harpin (8 $\mu\text{gm/ml}$) seemed enhanced in their stem growth more than those treated with either higher or lower
10 doses. Treatment with the medium dose of harpin resulted in greater growth under both growing conditions.

15 **Example 9 - Effect of Spraying Tomatoes With a Cell-Free Elicitor Preparation Containing the *Erwinia amylovora* Harpin**

Marglobe tomato plants were sprayed with harpin preparation (from *E. coli* DH5 α (pCPP2139)) or vector preparation (from *E. coli* DH5 α (pCPP50)) with added BSA
20 protein as control 8 days after transplanting. The control vector preparation contained, per ml, 33.6 μl of BSA (10 mg/ml) to provide about the same amount of protein as contained in the pCPP2139 preparation due to harpin. Dilutions of 1:50 (8.0 $\mu\text{g/ml}$), 1:100
25 (4.0 $\mu\text{g/ml}$), and 1:200 (2.0 $\mu\text{g/ml}$) were prepared and sprayed on the plants to runoff with an electricity-powered atomizer. Fifteen uniform appearing plants per treatment were chosen randomly and assigned to treatment. The plants were maintained at 28°C in a controlled
30 environment chamber before and after treatment.

Overall heights were measured several times after treatment from the surface of soil to the top of the plant. The tops of the tomato plants were weighed immediately after cutting the stems near the surface of
35 the soil.

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Treatments: (Dilutions and harpin content)

| | | | |
|----|--------------|-------|-------------------------|
| 1. | Harpin | 1:50 | (8.0 $\mu\text{g/ml}$) |
| 2. | Harpin | 1:100 | (4.0 $\mu\text{g/ml}$) |
| 3. | Harpin | 1:200 | (2.0 $\mu\text{g/ml}$) |
| 4. | Vector + BSA | 1:50 | (0 harpin) |
| 5. | Vector + BSA | 1:100 | (0 harpin) |
| 6. | Vector + BSA | 1:200 | (0 harpin) |

Table 21 -Tomato plant height (cm) 1 day after spray treatment

| Treat | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Mean |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| H 50 | 5.4 | 5.0 | 5.6 | 5.0 | 5.2 | 4.8 | 5.0 | 5.2 | 5.4 | 5.0 | 5.6 | 4.8 | 4.6 | 5.0 | 5.8 | 5.16 |
| H 100 | 5.0 | 5.2 | 5.0 | 5.4 | 5.4 | 5.0 | 5.2 | 4.8 | 5.6 | 5.2 | 5.4 | 5.0 | 4.8 | 5.0 | 5.2 | 5.15 |
| H 200 | 5.0 | 4.6 | 5.4 | 4.6 | 5.0 | 5.2 | 5.4 | 4.8 | 5.0 | 5.2 | 5.4 | 5.2 | 5.0 | 5.2 | 5.0 | 5.13 |
| | | | | | | | | | | | | | | | | |
| V 50 | 5.2 | 4.6 | 4.8 | 5.0 | 5.6 | 4.8 | 5.0 | 5.2 | 5.6 | 5.4 | 5.2 | 5.8 | 5.0 | 4.8 | 5.2 | 5.15 |
| V 100 | 5.2 | 4.8 | 5.2 | 5.0 | 5.6 | 4.8 | 5.4 | 5.2 | 5.0 | 4.8 | 5.0 | 4.8 | 5.6 | 5.2 | 5.4 | 5.13 |
| V 200 | 5.2 | 5.4 | 5.0 | 5.4 | 5.2 | 5.4 | 5.0 | 5.2 | 5.4 | 5.2 | 4.6 | 4.8 | 5.2 | 5.0 | 5.4 | 5.16 |

Table 22 -Tomato plant height (cm) 15 days after spray treatment

| Treat | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Mean |
|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| H 50 | 22.0 | 21.0 | 22.0 | 21.5 | 23.0 | 22.0 | 23.5 | 25.0 | 22.0 | 20.5 | 21.0 | 23.5 | 22.0 | 22.5 | 21.0 | 22.2 |
| H 100 | 26.0 | 26.5 | 27.0 | 29.0 | 27.5 | 26.0 | 28.0 | 29.0 | 28.5 | 26.0 | 27.5 | 28.0 | 28.0 | 29.0 | 26.0 | 27.5 |
| H 200 | 24.5 | 26.0 | 25.0 | 26.0 | 26.5 | 27.5 | 28.5 | 28.0 | 26.0 | 24.0 | 26.5 | 24.5 | 26.0 | 24.0 | 27.5 | 26.0 |
| | | | | | | | | | | | | | | | | |
| V 50 | 23.5 | 21.5 | 20.5 | 22.5 | 20.5 | 21.0 | 22.0 | 23.5 | 22.0 | 20.5 | 22.0 | 21.0 | 20.5 | 22.5 | 21.5 | 21.7 |
| V 100 | 22.5 | 21.0 | 20.5 | 23.0 | 22.0 | 20.0 | 20.5 | 20.0 | 21.0 | 22.0 | 23.0 | 20.0 | 22.0 | 21.0 | 22.5 | 21.4 |
| V 200 | 21.5 | 20.5 | 23.5 | 20.5 | 22.0 | 22.0 | 22.5 | 20.0 | 22.0 | 23.5 | 23.5 | 22.0 | 20.0 | 23.0 | 21.0 | 21.8 |

Table 23 -Tomato plant height (cm) 21 days after spray treatment

| Treat | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Mean |
|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| H 50 | 28.5 | 28.0 | 27.5 | 26.0 | 27.0 | 28.5 | 28.5 | 29.0 | 30.0 | 28.5 | 29.0 | 27.0 | 28.5 | 28.0 | 27.0 | 28.1 |
| H 100 | 37.0 | 38.0 | 37.5 | 39.0 | 37.0 | 38.5 | 36.0 | 38.0 | 37.0 | 38.5 | 37.0 | 36.0 | 37.0 | 37.0 | 38.5 | 37.5 |
| H 200 | 34.5 | 34.0 | 36.0 | 33.5 | 32.0 | 34.5 | 32.5 | 34.0 | 32.0 | 36.5 | 30.5 | 32.0 | 30.0 | 32.5 | 34.0 | 33.2 |
| | | | | | | | | | | | | | | | | |
| V 50 | 30.0 | 28.0 | 28.0 | 28.5 | 30.0 | 27.0 | 26.5 | 28.0 | 29.5 | 28.5 | 26.5 | 28.5 | 27.0 | 29.5 | 28.5 | 28.3 |
| V 100 | 28.0 | 27.5 | 30.0 | 29.5 | 28.5 | 29.0 | 30.0 | 26.5 | 27.5 | 28.0 | 30.0 | 29.0 | 28.5 | 28.0 | 29.5 | 28.6 |
| V 200 | 28.5 | 30.5 | 27.0 | 29.0 | 28.5 | 27.5 | 29.0 | 30.0 | 28.0 | 28.5 | 29.0 | 30.5 | 27.5 | 28.5 | 28.0 | 28.7 |

Table 24 -Mean Height of Tomato Plants After Spraying

| Treatment (Dil. & harpin) | Mean height of tomato plants (cm) | | | |
|---------------------------|-----------------------------------|--------|--------|--|
| | Days After Treatment | | | |
| | Day 1 | Day 11 | Day 14 | |
| Harpin 1:50 | (8.0 µg/ml) | 22.2 | 28.1 | |
| Harpin 1:100 | (4.0 µg/ml) | 27.5 | 37.5 | |
| Harpin 1:200 | (2.0 µg/ml) | 26.0 | 33.2 | |
| Vector + BSA 1:50 | (0) | 21.7 | 28.5 | |
| Vector + BSA 1:100 | (0) | 21.4 | 28.6 | |
| Vector + BSA 1:200 | (0) | 21.8 | 28.7 | |

Table 25 - Fresh Weight of Tomato Plants (g/plant)
21 Days After Spray Treatment

| Treat | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Mean |
|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| H 50 | 65.4 | 60.3 | 58.9 | 73.2 | 63.8 | 70.1 | 58.4 | 60.1 | 62.7 | 55.6 | 58.3 | 68.9 | 58.2 | 64.2 | 56.4 | 62.3 |
| H 100 | 84.3 | 68.8 | 74.6 | 66.7 | 78.5 | 58.9 | 76.4 | 78.6 | 84.8 | 78.4 | 86.4 | 66.5 | 76.5 | 82.4 | 80.5 | 76.2 |
| H 200 | 80.1 | 76.5 | 68.4 | 79.5 | 64.8 | 79.6 | 76.4 | 80.2 | 66.8 | 72.5 | 78.8 | 72.3 | 62.8 | 76.4 | 73.2 | 73.9 |
| | | | | | | | | | | | | | | | | |
| V 50 | 64.0 | 56.8 | 69.4 | 72.3 | 56.7 | 66.8 | 71.2 | 62.3 | 61.0 | 62.5 | 63.4 | 58.3 | 72.1 | 67.8 | 67.0 | 64.7 |
| V 100 | 62.8 | 58.4 | 70.2 | 64.2 | 58.1 | 72.7 | 68.4 | 53.6 | 67.5 | 66.3 | 59.3 | 68.2 | 71.2 | 65.2 | 59.2 | 64.4 |
| V 200 | 64.2 | 59.6 | 70.2 | 66.6 | 64.3 | 60.4 | 60.8 | 56.7 | 71.8 | 60.6 | 63.6 | 58.9 | 68.3 | 57.2 | 60.0 | 62.9 |

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A single spray of tomato seedlings with harpin, in general, resulted in greater subsequent growth than spray treatment with the control (vector) preparation, which had been supplemented with BSA protein. Enhanced growth in the harpin-treated plants was seen in both plant height and fresh weight measurements. Of the three concentrations tested, the two lower ones resulted in more plant growth (based on either measure) than the higher dose (8.0 $\mu\text{g/ml}$). There was little difference in the growth of plants treated with the two lower (2 and 4 $\mu\text{g/ml}$) concentrations. Components of the lysed cell preparation from the strain *E. coli* DH5 α (pCPP50), which harbors the vector of the *hrpN* gene in *E. coli* strain DH5 α (pCPP2139), do not have the same growth-promoting effect as the harpin-containing preparation, even though it is supplemented with BSA protein to the same extent as the DH5 α (pCPP2139) preparation, which contains large amounts of harpin protein. Thus, this experiment demonstrates that harpin is responsible for enhanced plant growth.

Example 10 - Early Coloration and Early Ripening of Small Fruits

A field trial was conducted to evaluate the effect of hypersensitive response elicitor ("harpin") treatment on yield and ripening parameters of raspberry cv. Canby. Established plants were treated with harpin at 2.5 mg/100 square feet in plots 40 feet long x 3 feet wide (1 plant wide), untreated ("Check"), or treated with the industry standard chemical Ronilan at recommended rates ("Ronilan"). Treatments were replicated four times and arranged by rep in an experimental field site. Treatments were made beginning at 5-10% bloom followed by two applications at 7-10 day intervals. The first two harvests were used to evaluate disease control and fruit

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yield data was collected from the last two harvests. Observations indicated harpin-treated fruits were larger and exhibited more redness than untreated fruits, indicating ripening was accelerated by 1-2 weeks. The
 5 number of ripe fruits per cluster bearing a minimum of ten fruits was determined at this time and is summarized in Table 26. Harpin treated plots had more ripe fruits per 10-berry cluster than either the check or Ronilan
 10 treatments. Combined yields from the last two harvests indicated increased yield in harpin and Ronilan treated plots over the untreated control (Table 27).

Table 26 - Number of Ripe Raspberry Fruits Per Clusters With Ten Berries or More on June 20, 1996.

| <u>Treatment</u> | <u>Ripe fruit/10 berry clusters</u> | <u>% of Control</u> |
|------------------|-------------------------------------|---------------------|
| Check | 2.75 | 100.0 |
| Ronilan | 2.75 | 100.0 |
| Harpin | 7.25 | 263.6 |

Table 27 - Mean Raspberry Fruit Yield by Weight (lbs.) Combined in Last Two Harvest.

| <u>Treatment</u> | <u>Total Yield</u> | <u>% of Control</u> |
|------------------|--------------------|---------------------|
| Check | 32.5 | 100.0 |
| Ronilan | 37.5 | 115.4 |
| Harpin | 39.5 | 121.5 |

Example 11 - Growth Enhancement For Snap Beans

Snap beans of the variety Bush Blue Lake were treated by various methods, planted in 25-cm-d plastic
 5 pots filled with commercial potting mix, and placed in an open greenhouse for the evaluation of growth parameters. Treatments included untreated bean seeds ("Check"), seeds treated with a slurry of 1.5% methyl cellulose prepared with water as diluent ("M/C"), seeds treated with 1.5%
 10 methyl cellulose followed by a foliar application of hypersensitive response elicitor ("harpin") at 0.125

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mg/ml ("M/C+H"), and seeds treated with 1.5% methyl cellulose plus harpin spray dried at 5.0 µg harpin per 50 seeds followed by a foliar application of harpin at 0.125 mg/ml ("M/C-SD+H"). Seeds were sown on day 0, planted 3 per pot, and thinned to 1 plant per pot upon germination. Treatments were replicated 10 times and randomized by rep in an open greenhouse. Bean pods were harvested after 64 days, and fresh weights of bean pods of marketable size (>10 cm x 5 cm in size) were collected as yield. Data were analyzed by analysis of variance with Fisher's LSD used to separate treatment means.

Table 28 - Effect of *Erwinia amylovora* Harpin Treatment by Various Methods on Yield of Market Sized Snap Bean Pods

| | <u>Treatment</u> | <u>Marketable Yield, g¹</u> | <u>% of Untreated (Check)</u> |
|----|------------------|--|-------------------------------|
| 20 | M/C-SD+H | 70.6 a | 452 |
| | M/C-H | 58.5 ab | 375 |
| | M/C | 46.3 bc | 297 |
| | M/C+H | 42.3 bc | 271 |
| | M/C-SD | 40.0 cd | 256 |
| 25 | Check | 15.6 e | 100 |

¹ Marketable yield included all bean pods 10 cm x 0.5 cm or larger. Means followed by the same letter are not significantly different at P=0.05 according to Fisher's LSD.

As shown in Table 28, the application of *Erwinia amylovora* harpin by various methods of application resulted in an increase in the yield of marketable size snap bean pods. Treatment with methyl cellulose alone also results in an increase in bean yield but was substantially increased when combined with harpin as seed (spray dried) and foliar treatments.

40

Example 12 - Yield Increase in Cucumbers from Foliar Application of HP-1000™ to Cucumbers.

Cucumber seedlings and transplants were treated with foliar sprays of HP-1000™ (EDEN Bioscience, Bothell,

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Washington) (*Erwinia amylovora* hypersensitive response elicitor formulation) at rates of 15, 30, or 60 $\mu\text{g/ml}$ active ingredient (a.i.). The first spray was applied when the first true leaves were fully expanded. The

5 second application was made 10 days after the first spray. All sprays were applied using a back-pack sprayer, and an untreated control (UTC) was also included in the trial. Three days after the second application of HP-1000™, ten plants from each treatment were

10 transplanted into randomized field plots replicated three times. This yielded a total of thirty plants per treatment. Seven days after transplanting, a third foliar spray of HP-1000™ was applied. Although severe drought followed resulting in significant water stress, a total

15 of six harvests were made following a standard commercial harvesting pattern. The total weight of fruit harvested from each treatment is presented in Table 29. Results indicate that plants treated with HP-1000™ at rates of

20 15 and 30 $\mu\text{g/ml}$ yielded significantly more fruit than the UTC. Plants treated with HP-1000™ yielded a moderate yield increase. These results indicated that HP-1000™ treated plants were significantly more tolerant to drought stress conditions than untreated plants.

25 Table 29 - Increase yield of cucumbers after treatment with HP-1000™

| 30 | Treatment | Rate ¹ | Yield, ² lbs./10 plants | % above UTC |
|----|-----------|---------------------|------------------------------------|-------------|
| | UTC | --- | 9.7 a | --- |
| | HP-1000™ | 15 $\mu\text{g/ml}$ | 25.4 b | 161.4 |
| | HP-1000™ | 30 $\mu\text{g/ml}$ | 32.6 c | 236.4 |
| | HP-1000™ | 60 $\mu\text{g/ml}$ | 11.2 a | 15.9 |

35
40 ¹Active ingredient (a.i.). ²Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

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Example 13 - Yield Increase in Cotton from Treatment with HP-1000™

Cotton was planted in four, 12 x 20 foot replicate field plots in a randomized complete block (RCB) field trial. Plants were treated with HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation), HP-1000™+Pix° (Pix° (BASF Corp., Mount Olive, N.J.) is a growth regulator applied to keep cotton plants compact in height) or Early Harvest° (Griffen Corp., Valdosta, Ga.) (a competitive growth enhancing agent). An untreated control (UTC) was also included in the trial. Using a back-pack sprayer, foliar applications were made of all treatments at three crop growth stages; first true leaves, pre-bloom, and early bloom. All fertilizers and weed control products were applied according to conventional farming practices for all treatments. The number of cotton bolls per plant ten weeks before harvest was significantly higher for the HP-1000™ treated plants compared to other treatments. By harvest, HP-1000™ treatment was shown to have a significantly increased lint yield (43%) compared to UTC (Table 30). When HP-1000™ was combined with Pix°, lint yield was increased 20% over UTC. Since Pix° is commonly applied to large acreages of cotton, this result indicates that HP-1000™ may be successfully tank-mixed with Pix°. Application of the competitive growth enhancing agent, Early Harvest° only produced a 9% increase in lint yield vs. UTC.

30

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Table 30 - Increased lint yield from cotton after treatment with HP-1000™, HP-1000™+Pix®, or Early Harvest®.

| | |
|----|--|
| 5 | ----- |
| | Treatment Rate ¹ Lint Yield (lbs./ac) % above |
| | UTC |
| | UTC --- 942.1 --- |
| 10 | Early Harvest® 2 oz./ac. 1,077.4* 14.3 |
| | HP-1000™+Pix® 40 µg/ml+8 oz./ac. 1,133.1* 20.4 |
| | HP-1000™ 40 µg/ml 1,350.0* 43.3 |
| | (*significant at P= 0.05) lsd = 122.4 |
| 15 | ----- |

¹Rates for HP-1000™ are for active ingredient (a.i.); rates for Early Harvest® and Pix® are formulated product.

20

Example 14 - Yield Increase of Chinese Egg Plant from Treatment with HP-1000™

Nursery grown Chinese egg plant seedlings were
 25 sprayed once with HP-1000™ at (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation)
 15, 30, or 60 µg/ml (a.i.), then transplanted into field plots replicated three times for each treatment. Two weeks after transplanting, a second application of
 30 HP-1000™ was made. A third and final application of HP-1000™ was applied approximately two weeks after the second spray. All sprays were applied using a back-pack sprayer; an untreated control (UTC) was also included in the trial. As the season progressed, a total of eight
 35 harvests from each treatment were made. Data from these harvests indicate that treatment with HP-1000™ resulted in greater yield of fruit per plant.

40

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Table 31 - Increased yield for Chinese egg plant after treatment with HP-1000™.

| | | | | |
|----|-----------|-------------|-------------------|-------------|
| 5 | Treatment | Rate (a.i.) | Yield(lbs./plant) | % above UTC |
| | UTC | -- | 1.45 | --- |
| | HP-1000™ | 15 µg/ml | 2.03 | 40.0 |
| | HP-1000™ | 30 µg/ml | 1.90 | 31.0 |
| 10 | HP-1000™ | 60 µg/ml | 1.95 | 34.5 |

15 Example 15 - Yield Increase of Rice From Treatment with HP-1000™

Rice seedlings were transplanted into field plots replicated three times, then treated with foliar sprays of HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation) at three different rates using a back-pack sprayer. An untreated control (UTC) was also included in the trial. The first application of HP-1000™ was made one week after transplanting, the second three weeks after the first. A third and final spray was made just before rice grains began to fill the heads. Results at harvest demonstrated that foliar applications of HP-1000™ at both 30 and 60 µg/ml significantly increased yield by 47 and 56%, respectively (Table 32).

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Table 32 - Increase yield of rice after foliar treatment with HP-1000™.

| | | | | |
|----|-----------|-------------|-------------------------------|-------------|
| 5 | Treatment | Rate (a.i.) | Yield ¹ (lbs./ac.) | % above UTC |
| | UTC | --- | 3,853 a | --- |
| | HP-1000™ | 15 µg/ml | 5,265 ab | 35.9 |
| | HP-1000™ | 30 µg/ml | 5,710 b | 47.3 |
| 10 | HP-1000™ | 60 µg/ml | 6,043 b | 56.1 |

15 ¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Example 16 - Yield Increase of Soybeans From Treatment with HP-1000™

20 Soybeans were planted into randomized field plots replicated three times for each treatment. A back-pack sprayer was used to apply foliar sprays of HP-1000™ (EDEN Bioscience) (*Erwinia amylovora*

25 hypersensitive response elicitor formulation) and an untreated control (UTC) was also included in the trial. Three rates of HP-1000™ were applied beginning at four true leaves when plants were approximately eight inches tall. A second spray of HP-1000™ was applied ten days

30 after the first spray and a third spray ten days after the second. Plant height measured ten days after the first spray treatment indicated that application of HP-1000™ resulted in significant growth enhancement (Table 33). In addition, plants treated with HP-1000™ at

35 the rate of 60 µg/ml began to flower five days earlier than the other treatments. Approximately ten days after application of the third spray, the number of soybean pods per plant was counted from ten randomly selected plants per replication. These results indicated that the

40 growth enhancement from treatment with HP-1000™ resulted in significantly greater yield (Table 34).

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Table 33 - Increased plant height of soybeans after foliar treatment with HP-1000™.

| | | | | |
|----|-----------|-------------|------------------------------|-------------|
| 5 | Treatment | Rate (a.i.) | Plant Ht. ¹ (in.) | % above UTC |
| | UTC | --- | 12.2 a | --- |
| | HP-1000™ | 15 µg/ml | 13.2 b | 8.3 |
| | HP-1000™ | 30 µg/ml | 14.1 c | 16.2 |
| 10 | HP-1000™ | 60 µg/ml | 14.3 c | 17.3 |

Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Table 34 - Increased pod set of soybeans after foliar treatment with HP-1000™.

| | | | | |
|----|-----------|-------------|-----------------------------|-------------|
| 20 | Treatment | Rate (a.i.) | No. Pods/plant ¹ | % above UTC |
| | UTC | --- | 41.1 a | --- |
| | HP-1000™ | 15 µg/ml | 45.4 ab | 10.4 |
| 25 | HP-1000™ | 30 µg/ml | 47.4 b | 15.4 |
| | HP-1000™ | 60 µg/ml | 48.4 b | 17.7 |

Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Example 17 - Yield Increase of Strawberries From Treatment with HP-1000™

35 Two field trials with HP-1000™ (EDEN Bioscience) (Erwinia amylovora hypersensitive response elicitor formulation) were conducted on two strawberry varieties, Camarosa and Selva. For each variety, a

40 randomized complete block (RCB) design was established having four replicate plots (5.33 x 10 feet) per treatment in a commercially producing strawberry field. Within each plot, strawberry plants were planted in a

45 double row layout. An untreated control (UTC) was also included in the trial. Before applications began, all plants were picked clean of any flowers and berries.

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Sprays of HP-1000™ at the rate of 40 µg/ml were applied as six weekly using a back-pack sprayer. Just prior to application of each spray, all ripe fruit from each treatment was harvested, weighed, and graded according to commercial standards. Within three weeks of the first application of HP-1000™ to Selva strawberry plants, growth enhancement was discernible as visibly greater above-ground biomass and a more vigorous, greener and healthier appearance. After six harvests (i.e. the scheduled life-span for these plants), all yield data were summed and analyzed. For the Camarosa variety, yield of marketable fruit from HP-1000™ treated plants was significantly increased (27%) over the UTC when averaged over the last four pickings (Table 35).

Significant differences between treatments were not apparent for this variety for the first two pickings. The Selva variety was more responsive to the growth enhancing effects from treatment with HP-1000™; Selva strawberry plants yielded a statistically significant 64% more marketable fruit vs. the UTC when averaged over six pickings (Table 35).

Table 35 - Increased yield of strawberries after foliar treatment with HP-1000™.

| Treatment | Rate (a.i.) | Yield ¹ (lbs./rep) | % above |
|-------------------|-------------|-------------------------------|---------|
| UTC | | | |
| Variety: Camarosa | | | |
| UTC | --- | 1.71 a | --- |
| HP-1000™ | 40 µg/ml | 2.17 b | 27 |
| Variety: Selva | | | |
| UTC | --- | 0.88 a | --- |
| HP-1000™ | 40 µg/ml | 1.44 b | 64 |

¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

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Example 18 - Earlier Maturity and Increased Yield of Tomatoes from Treatment with HP-1000™

Fresh market tomatoes (var. Solar Set) were grown in plots (2 x 30 feet) replicated 5 times in a randomized complete block (RCB) field trial within a commercial tomato production field. Treatments included HP-1000™ (EDEN Bioscience) (Erwinia amylovora hypersensitive response elicitor formulation), an experimental competitive product (Actigard™ (Novartis, Greensboro, N.C.)) and a chemical standard (Kocide® (Griffen Corp., Valdosta, GA)) + Maneb® (DuPont Agricultural Products, Wilmington, D.E.)) for disease control. The initial application of HP-1000™ was made as a 50 ml drench (of 30 µg/ml a.i.) poured directly over the seedling immediately after transplanting. Thereafter, eleven weekly foliar sprays were applied using a back-pack sprayer. The first harvest from all treatments was made approximately six weeks after transplanting and only fully red, ripe tomatoes were harvested from each treatment. Results indicated that HP-1000™ treated plants had a significantly greater amount of tomatoes ready for the first harvest (Table 36). The tomatoes harvested from the HP-1000™ treated plants were estimated to be 10-14 days ahead of other treatments.

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Table 36 - Increased yield of tomatoes at first harvest after foliar treatment with of HP-1000™.

| 5 | Treatment | Rate (a.i.) ¹ | Yield ² (lbs./rep) | % above |
|----|-----------|--------------------------|-------------------------------|---------|
| | UTC | | | |
| | UTC | --- | 0.61 a | --- |
| | HP-1000™ | 30 µg/ml | 2.87 b | 375 |
| 10 | Actigard™ | 14 g/ac | 0.45 a | -25.1 |
| | Kocide®+ | 2 lbs./ac. | 0.31 a | -49.1 |
| | Maneb® | 1 lb./ac | | |

¹Rates for Kocide® and Maneb® are for formulated product. ²Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Example 19 - Earlier Flowering and Growth Enhancement of Strawberries From Treatment with HP-1000™ When Planted in Non-fumigated Soil.

Strawberry plants ("plugs" and "bare-root"), cv. *Commander* were transplanted into plots (2 x 30 feet) replicated 5 times in a randomized complete block field trial. Approximately sixty individual plants were transplanted into each replicate. Treatments applied in this field trial are listed below:

| 35 | <u>Treatment</u> | <u>Application method</u> |
|----|-----------------------------------|--|
| | HP-1000™ (plug plants) | 50-ml drench solution of HP-1000™ (EDEN Bioscience) (<i>Erwinia amylovora</i> hypersensitive response elicitor formulation) at 40 µg/ml (a.i.) poured directly over the individual plants immediately after transplanting into non-fumigated soil ¹ , followed by foliar applications of HP-1000™ at 40 µg/ml every 14 days. |
| 45 | HP-1000™ 40 (bare-root plants) | root soak in solution of HP-1000™ at µg/ml (a.i.) for 1 hour, immediately before transplanting into non-fumigated |

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soil,¹ followed by foliar applications of HP-1000™ at 40 µg/ml every 14 days.

- 5 methyl bromide/
chlorpicrin soil fumigation at 300 lbs./ac via
75/25 injection prior to transplanting, no
HP-1000™ treatments applied.
- 10 Telone/chlorpicrin soil fumigation at 45 gal./ac via
70/30 injection prior to transplanting, no
HP-1000™ treatments applied.
- untreated control no fumigation, no HP-1000™ treatments
(UTC)
- 15 ¹Non-fumigated soil had been cropped to vetch for the two previous
years.
- 20 Transplanting was done in late fall when cool weather
tended to slow plant growth. Two weeks after
transplanting, the first foliar application of HP-1000™
was made at 40 µg/ml (a.i.) with a back-pack sprayer.
Three weeks after transplanting, preliminary results were
25 gathered comparing HP-1000™ treatment against methyl
bromide and UTC by counting the number of flowers on all
strawberry "plug" plants in each replication. Since
flowering had not yet occurred in the "bare-root" plants,
each plant in replicates for this treatment was assessed
30 for early leaf growth by measuring the distance from leaf
tip to stem on the middle leaf of 3-leaf cluster.
Results (Tables 37 and 38) indicated that treatment with
HP-1000™ provided early enhanced flower growth and leaf
size for "plug" and "bare-root" strawberry plants,
35 respectively.

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Table 37 - Earlier flowering of "plug" strawberry transplants after foliar treatment with HP-1000™.

| | | | | |
|----|---------------------------------|-------------|------------------------------|---------|
| 5 | ----- | | | |
| | Treatment | Rate (a.i.) | No. flowers/rep ¹ | % above |
| | UTC | | | |
| | UTC | --- | 2.0a | --- |
| 10 | HP-1000™ | 40 µg/ml | 7.5 b | 275 |
| | Methyl bromide/ chloropicrin | 300 lbs./ac | 5.3 b | 163 |
| | ----- | | | |

15 ¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

20 Table 38 - Increased leaf growth in "bare-root" strawberry transplants after foliar treatment with HP-1000™.

| | | | | |
|----|-----------|-------------|--------------------------------|---------|
| 25 | ----- | | | |
| | Treatment | Rate (a.i.) | Leaf length ¹ (in.) | % above |
| | UTC | | | |
| | UTC | --- | 1.26 a | --- |
| 30 | HP-1000™ | 40 µg/ml | 1.81 b | 44 |
| | ----- | | | |

35 ¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Example 20 - Early Growth Enhancement of Jalapeño Peppers from Application of HP-1000™

40 Jalapeño pepper (cv. Mittlya) transplants were treated with a root drench of HP-1000 (EDEN Bioscience) (Erwinia amylovora hypersensitive response elicitor formulation) (30 µg/ml a.i.) for 1 hour, then

45 transplanted into randomized field plots replicated four times. An untreated control (UTC) was also included. Beginning 14 days after transplanting, treated plants received three foliar sprays of HP-1000™ at 14 day

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intervals using a back-pack sprayer. One week after the third application of HP-1000™ (54 days after transplanting), plant height was measured from four randomly selected plants per replication. Results from these measurements indicated that the HP-1000™ treated plants were approximately 26% taller than the UTC plants (Table 39). In addition, the number of buds, flowers or fruit on each plant was counted. These results indicated that the HP-1000™ treated plants had over 61% more flowers, fruit or buds compared to UTC plants (Table 40).

Table 39 - Increased plant height in Jalapeño peppers after treatment with HP-1000™.

| | | | | |
|----|-----------|-------------|-----------------------------|-------------|
| 15 | ----- | | | |
| | Treatment | Rate (a.i.) | Plant Ht.(in.) ¹ | % above UTC |
| | UTC | --- | a7.0 | --- |
| 20 | HP-1000™ | 30 µg/ml | 8.6 b | 23.6 |

¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Table 40 - Increased number of flowers, fruit or buds in Jalapeño peppers after treatment with HP-1000™.

| | | | | |
|----|-----------|-------------|--|---------|
| 35 | | | No. flowers, fruit or buds/plant ¹ | % above |
| | Treatment | Rate (a.i.) | | |
| | UTC | | | |
| | UTC | --- | 20.6 a | --- |
| | HP-1000™ | 30 µg/ml | 12.8 b | 61.3 |

¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

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**Example 21 - Growth Enhancement of Tobacco from
Application of HP-1000™**

Tobacco seedlings were transplanted into
5 randomized field plots replicated three times. A foliar
spray of HP-1000™ (EDEN Bioscience) (*Erwinia amylovora*
hypersensitive response elicitor formulation) was applied
after transplanting at one of three rates: 15, 30, or 60
μg/ml a.i. Sixty days later, a second foliar application
10 of HP-1000 was made. Two days after the second
application, plant height, number of leaves per plant,
and the leaf size (area) were measured from ten, randomly
selected plants per treatment. Results from these
measurements indicated treatment with HP-1000™ enhanced
15 tobacco plant growth significantly (Tables 41, 42, and
43). Plant height was increased by 6-13%, while plants
treated with HP-1000™ at 30 and 60 μg/ml averaged just
over 1 more leaf per plant than UTC. Most significantly,
however, treatment with HP-1000™ at 15, 30, and 60 μg/ml
20 resulted in corresponding increases in leaf area.
Tobacco plants with an extra leaf per plant and an
increase in average leaf size (area) represent a
commercially significant response.

25 Table 41 - Increased plant height in tobacco after
treatment with HP-1000™.

30

| ----- | | | |
|-------------|-------------|----------------|-------------|
| Treatment | Rate (a.i.) | Plant Ht. (cm) | % above UTC |
| UTC | --- | 72.0 | --- |
| HP-1000™ | 15 μg/ml | 76.4 | 5.3 |
| HP-1000™ | 30 μg/ml | 79.2 | 9.0 |
| 35 HP-1000™ | 60 μg/ml | 81.3 | 6.9 |
| ----- | | | |

40

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Table 42 - Increased number of tobacco leaves per plant after treatment with HP-1000™.

| | | | | |
|----|-----------|-------------|---------------------------|-------------|
| 5 | Treatment | Rate (a.i.) | Leaves/plant ¹ | % above UTC |
| | UTC | --- | 16.8 | --- |
| | HP-1000™ | 15 µg/ml | 17.4 | 3.6 |
| | HP-1000™ | 30 µg/ml | 18.1 | 7.7 |
| 10 | HP-1000™ | 60 µg/ml | 17.9 | 6.5 |

Table 43 - Increased leaf area in tobacco after treatment with HP-1000™.

| | | | | |
|----|-----------|-------------|------------------------------|-------------|
| 20 | Treatment | Rate (a.i.) | Leaf area (cm ²) | % above UTC |
| | UTC | --- | 1,246 | --- |
| | HP-1000™ | 15 µg/ml | 1,441 | 16 |
| | HP-1000™ | 30 µg/ml | 1,543 | 24 |
| 25 | HP-1000™ | 60 µg/ml | 1,649 | 32 |

30 Example 22 - Growth Enhancement of Winter Wheat from Application of HP-1000™

Winter wheat seed was "dusted" with dry HP-1000™ (EDEN Bioscience) (Erwinia amylovora hypersensitive response elicitor formulation) powder at the rate of 3 ounces of formulated product (3% a.i.) per 100 lbs. seed, then planted using conventional seeding equipment into randomized test plots 11.7 feet by 100 feet long. Additional treatments included a seed "dusting" with HP-1000™ powder (3% a.i.) at 1 oz. formulated product per 100 lbs. seed, a seed-soak in a solution of HP-1000™ at a concentration of 20 µg/ml, a.i., for four hours, then air-dried before planting, a standard chemical (Dividend®) fungicide "dusting", and an untreated control (UTC). Eight days after planting,

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HP-1000™ treated seeds began to emerge, whereas the UTC and chemical standard-treated seed did not emerge until approximately 14 days after planting, the normal time expected. At 41 days after planting, seedlings were removed from the ground and evaluated. Root mass for wheat treated with HP-1000™ as a "dusting" at 3 oz./100 lb. was visually inspected and judged to be approximately twice as great as any of the other treatments.

Following the field trial, a greenhouse experiment was designed to gain confirmation of these results. Treatments included wheat seed dusted with dry HP-1000™ (10% a.i.) at a rate of 3 ounces per 100 lbs. of seed, seed soaking of HP-1000™ in solution concentration of 20 mg/ml for four hours before planting, and an untreated control (UTC). Wheat seeds from each treatment were planted at the rate of 25 seeds per pot, with five pots serving as replicates for each treatment. Fifteen days after planting, ten randomly selected seedlings from each treatment pot were removed, carefully cleaned, and measured for root length. Since the above-ground portion of individual seedlings did not exhibit any treatment effect, increased root growth from treatment with HP-1000™ did not influence the selection of samples. The increase in root growth from either HP-1000™ treatment was significantly greater than UTC (Table 49); however, the seed dusting treatment appeared to give slightly better results.

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Table 44 - Increased root growth in wheat seedlings after treatment with HP-1000™.

| | | | | |
|----|-----------------------|----------------|--------------------------------|-------------|
| 5 | Treatment | Rate | Root length. (cm) ¹ | % above UTC |
| | UTC | --- | 35.6 a | --- |
| | HP-1000™ (dusting) | 3 oz./100 lbs. | 41.0 b | 17.4 |
| 10 | HP-1000™ (soaking) | 20 µg/ml | 40.8 b | 14.6 |

15 ¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

20 **Example 23 - Growth Enhancement of Cucumbers from Application of HP-1000™**

A field trial of commercially produced cucumbers consisted of four treatments, HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation) at two rates (20 or 40 µg/ml), a chemical standard for disease control (Bravo® (Zeneca Ag Products, Wilmington, Del.) +Maneb®) and an untreated control (UTC). Each treatment was replicated four times in 3 x 75 foot plots with a plant spacing of approximately 2 feet for each treatment. Foliar sprays of HP-1000™ were applied beginning at first true leaf and repeated at 14 day intervals until the last harvest for a total of six applications. The standard fungicide mix was applied every seven days or sooner if conditions warranted. Commercial harvesting began approximately two months after first application of HP-1000™ (after five sprays of HP-1000™ had been applied), and a final harvest was made approximately 14 days after the first harvest.

Results from the first harvest indicated that treatment with HP-1000™ enhanced the average cucumber yield by increasing the total number of cucumbers

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harvested and not the average weight of individual cucumbers (Tables 45-47). The same trend was noted at the final harvest (Tables 48-49). It was commercially important that the yield increase resulting from

5 treatment with HP-1000™ was not achieved by significantly increasing average cucumber size.

10 Table 45 - Increased cucumber yield after treatment with HP-1000™, first harvest.

| ----- | | | |
|-------------|-------------|------------------------------|-------------|
| Treatment | Rate (a.i.) | Yield/trt ¹ (kg.) | % above UTC |
| 15 UTC | --- | 10.0 a | --- |
| Bravo+Maneb | label | 10.8 a | 8.4 |
| HP-1000™ | 20 µg/ml | 12.3 ab | 22.8 |
| HP-1000™ | 40 µg/ml | 13.8 b | 38.0 |

20

¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

25

Table 46 - Increased number of fruit in cucumbers after treatment with HP-1000™, first harvest.

| ----- | | | |
|-------------|-------------|----------------------------|-------------|
| Treatment | Rate (a.i.) | No. fruit/trt ¹ | % above UTC |
| 35 UTC | --- | 24.5 a | --- |
| Bravo+Maneb | label | 27.6 ab | 12.8 |
| HP-1000™ | 20 µg/ml | 31.2 b | 27.0 |
| HP-1000™ | 40 µg/ml | 34.3 b | 39.8 |

40 ¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

45

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Table 47 - Average weight of cucumbers after treatment with HP-1000™, first harvest.

| | Treatment | Rate (a.i.) | Weight/fruit(g) | % change vs. |
|----|-------------|-------------|-----------------|--------------|
| 5 | UTC | | | |
| | UTC | --- | 406 | --- |
| | Bravo+Maneb | label | 390 | -4 |
| 10 | HP-1000™ | 20 µg/ml | 395 | -3 |
| | HP-1000™ | 40 µg/ml | 403 | -1 |

15

Table 48 - Increased cucumber yield after treatment with HP-1000™, third harvest.

| | Treatment | Rate (a.i.) | Yield/trt ¹ (kg.) | % above UTC |
|----|-------------|-------------|------------------------------|-------------|
| 20 | UTC | --- | 17.5 a | --- |
| | Bravo+Maneb | label | 14.0 b | -20.1 |
| 25 | HP-1000™ | 20 µg/ml | 20.1 a | 15.3 |
| | HP-1000™ | 40 µg/ml | 20.2 a | 15.6 |

30 'Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

35 Table 49 - Increased number of fruit in cucumbers after treatment with HP-1000™, third harvest.

| | Treatment | Rate (a.i.) | No. fruit/trt ¹ | % change vs. |
|----|-------------|-------------|----------------------------|--------------|
| 40 | UTC | | | |
| | UTC | --- | 68.8 ab | --- |
| | Bravo+Maneb | label | 60.0 a | -12.7 |
| | HP-1000™ | 20 µg/ml | 82.3 b | 19.6 |
| 45 | HP-1000™ | 40 µg/ml | 85.3 b | 24.0 |

50 'Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Table 50 - Average weight of cucumbers after treatment with HP-1000™, third harvest.

| | | | | |
|----|-------------|-------------|-----------------|--------------|
| 5 | Treatment | Rate (a.i.) | Weight/fruit(g) | % change vs. |
| | UTC | | | |
| | UTC | --- | 255 | --- |
| | Bravo+Maneb | label | 232 | -9 |
| 10 | HP-1000™ | 20 µg/ml | 247 | -3 |
| | HP-1000™ | 40 µg/ml | 237 | -7 |

15 **Example 24 - Harpin_{pss} from *Pseudomonas syringae* pv *syringae* Induces Growth Enhancement in Tomato**

20 To test if harpin_{pss} (i.e. the hypersensitive response elicitor from *Pseudomonas syringae* pv *syringae*) (He, S. Y., et al., "*Pseudomonas syringae* pv *syringae* Harpin_{pss}. A Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 25 73:1255-66 (1993), which is hereby incorporated by reference) also stimulates plant growth, tomato seeds (Marglobe variety) were sowed in 8 inches pots with artificial soil. 10 days after sowing, the seedlings were transplanted into individual pots. Throughout the 30 experiment, fertilizer, irrigation of water, temperature, and soil moisture were maintained uniformly among plants. 16 days after transplanting, the initial plant height was measured and the first application of harpin_{pss} was made, this is referred to as day 0. A second application was 35 made on day 15. Additional growth data was collected on day 10 and day 30. The final data collection on day 30 included both plant height and fresh weight.

The harpin_{pss} used for application during the experiment was produced by fermenting *E. coli* DH5 40 containing the plasmid with the gene encoding harpin_{pss} (i.e. *hrpZ*). The cells were harvested, resuspended in 5 mM potassium phosphate buffer, and disrupted by

- 81 -

sonication. The sonicated material was boiled for 5 minutes and then centrifugated for 10 min. at 10,000 rpm. The supernatant was considered as Cell-Free Elicitor Preparation (CFEP). 20 and 50 µg/ml harpin_{pss} solution was made with the same buffer used to make cell suspension. CFEP prepared from the same strain containing the same plasmid but without *hrpZ* gene was used as the material for control treatment.

The wetting agent, Pinene II (Drexel Chemical Co., Memphis, Tenn.) was added to the harpin_{pss} solution at the concentration of 0.1%, then harpin_{pss} was sprayed onto tomato plant until there was run off.

Table 51 shows that there was a significant difference between the harpin_{pss} treatment groups and the control group. Harpin_{pss} treated tomato increased more than 10% in height. The data supports the claim that harpin_{pss} does act similar to the hypersensitive response elicitor from *Erwinia amylovora*, in that when applied to tomato and many other species of plants, there is a growth enhancement effect. In addition to a significant increase of tomato height harpin_{pss}-treated tomato had more biomass, big leaves, early flower setting, and over all healthier appearance.

Table 51 - Harpin_{pss} enhances the growth of tomato plant

| Treatment | Plant Height (cm ¹) | | |
|--------------------|--|---------------|---------------|
| | Day 0 | Day 10 | Day 30 |
| CFEP Control | 8.5 ² (0.87) a ³ | 23.9 (1.90) a | 68.2 (8.60) a |
| Harpinpss 20 µg/ml | 8.8 (0.98) a | 27.3 (1.75) b | 74.2 (6.38) b |
| Harpinpss 50 µg/ml | 8.8 (1.13) a | 26.8 (2.31) b | 75.4 (6.30) b |

¹Plant height was measured to the nearest 0.5 cm. Day 0 refers to the day the initial plant heights were recorded and the first application was made.

²Means are given with SD in parenthesis (n=20 for all treatment groups).

5 ^bDifferent letters (a and b) indicates significant differences (P 0.05) among means. Differences were evaluated by ANOVA followed by Fisher LSD.

Although the invention has been described in
10 detail for the purpose of illustration, it is understood
that such detail is solely for that purpose, and
variations can be made therein by those skilled in the
art without departing from the spirit and scope of the
invention which is defined by the following claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Cornell Research Foundation, Inc.
- (ii) TITLE OF INVENTION: ENHANCEMENT OF GROWTH IN PLANTS
- (iii) NUMBER OF SEQUENCES: 10
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Nixon, Hargrave, Devans & Doyle LLP
 - (B) STREET: Clinton Square, P.O. Box 1051
 - (C) CITY: Rochester
 - (D) STATE: New York
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 14603
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 60/036,048
 - (B) FILING DATE: 27-JAN-1997
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Goldman, Michael L.
 - (B) REGISTRATION NUMBER: 30,727
 - (C) REFERENCE/DOCKET NUMBER: 19603/1502
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (716) 263-1304
 - (B) TELEFAX: (716) 263-1600

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 338 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
20      25      30
Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
35      40      45
Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
50      55      60
Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
65      70      75      80
Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
85      90      95
Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
100     105     110
Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
115     120     125
Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
130     135     140
Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
145     150     155     160
Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
165     170     175
Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
180     185     190
Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
195     200     205
Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
210     215     220
Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
225     230     235     240
Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
245     250     255
Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
260     265     270
Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
275     280     285
Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
290     295     300

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Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
 305 310 315 320
 Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
 325 330 335
 Asn Ala

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2141 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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|------------|-------------|------------|------------|------------|------------|------|
| CGATTTTACC | CGGGTGAACG | TGCTATGACC | GACAGCATCA | CGGTATTCGA | CACCGTTACG | 60 |
| GCGTTTATGG | CCGCGATGAA | CCGGCATCAG | GCGGCGCGCT | GGTCGCCGCA | ATCCGGCGTC | 120 |
| GATCTGGTAT | TTCAGTTTGG | GGACACCGGG | CGTGAACTCA | TGATGCAGAT | TCAGCCGGGG | 180 |
| CAGCAATATC | CCGGCATGTT | GCGCACGCTG | CTCGCTCGTC | GTTATCAGCA | GGCGGCAGAG | 240 |
| TGCGATGGCT | GCCATCTGTG | CCTGAACGGC | AGCGATGTAT | TGATCCTCTG | GTGGCCGCTG | 300 |
| CCGTCGGATC | CCGGCAGTTA | TCCGCAGGTG | ATCGAACGTT | TGTTTGAAC | GGCGGGAATG | 360 |
| ACGTTGCCGT | CGCTATCCAT | AGCACCGACG | GCGCGTCCGC | AGACAGGGAA | CGGACGCGCC | 420 |
| CGATCATTAA | GATAAAGGCG | GCTTTTTTTT | TTGCAAAACG | GTAACGGTGA | GGAACCGTTT | 480 |
| CACCGTCGGC | GTCACCTCAGT | AACAAGTATC | CATCATGATG | CCTACATCGG | GATCGGCGTG | 540 |
| GGCATCCGTT | GCAGATACTT | TTGCGAACAC | CTGACATGAA | TGAGGAAACG | AAATTATGCA | 600 |
| AATTACGATC | AAAGCGCACA | TCGGCGGTGA | TTTGGGCGTC | TCCGGTCTGG | GGCTGGGTGC | 660 |
| TCAGGGACTG | AAAGGACTGA | ATTCCGCGGC | TTCATCGCTG | GGTTCCAGCG | TGGATAAACT | 720 |
| GAGCAGCACC | ATCGATAAGT | TGACCTCCGC | GCTGACTTCG | ATGATGTTTG | GCGGCGCGCT | 780 |
| GGCGCAGGGG | CTGGGCGCCA | GCTCGAAGGG | GCTGGGGATG | AGCAATCAAC | TGGGCCAGTC | 840 |
| TTTCGGCAAT | GGCGCGCAGG | GTGCGAGCAA | CCTGCTATCC | GTACCGAAAT | CCGGCGGCGA | 900 |
| TGCGTTGTCA | AAAATGTTTG | ATAAAGCGCT | GGACGATCTG | CTGGGTCATG | ACACCGTGAC | 960 |
| CAAGCTGACT | AACCAGAGCA | ACCAACTGGC | TAATTCAATG | CTGAACGCCA | GCCAGATGAC | 1020 |
| CCAGGGTAAT | ATGAATGCGT | TCGGCAGCGG | TGTGAACAAC | GCACTGTCGT | CCATTCTCGG | 1080 |
| CAACGGTCTC | GGCCAGTCGA | TGAGTGGCTT | CTCTCAGCCT | TCTCTGGGGG | CAGGCGGCTT | 1140 |

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GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT      1200
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CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA      1320
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GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG      1440
CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA      1500
TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC      1560
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ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC      1800
GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC      1860
CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTCTATCC GCCCCTTTAG      1920
CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG      1980
GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC      2040
AAAATAGGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG      2100
GTTTCGTCATC ATCTTTCTCC ATCTGGGCGA CCTGATCGGT T                        2141

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 403 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser
1           5           10           15
Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln
20           25           30
Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn
35           40           45
Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
50           55           60

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Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu
 65 70 75 80
 Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
 85 90 95
 Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
 100 105 110
 Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
 115 120 125
 Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser
 130 135 140
 Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
 145 150 155 160
 Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
 165 170 175
 Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
 180 185 190
 Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
 195 200 205
 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
 210 215 220
 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
 225 230 235 240
 Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
 245 250 255
 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
 260 265 270
 Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
 275 280 285
 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
 290 295 300
 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
 305 310 315 320
 Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
 325 330 335
 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn
 340 345 350
 Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
 355 360 365
 Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
 370 375 380
 Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
 385 390 395 400
 Gly Ala Ala

- 88 -

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1288 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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| ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG | 180 |
| GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATACCGT CAATCAGCTG | 240 |
| GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG | 300 |
| GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA | 360 |
| GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTTTCG TGAACACGCT GGGCTCGAAA | 420 |
| GGCGGCAACA ATACCACTTC AACAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC | 480 |
| TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC | 540 |
| CCGATGCAGC AGCTGCTGAA GATGTTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG | 600 |
| CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC | 660 |
| GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG | 720 |
| CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC | 780 |
| GGTTCGTGCG TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG | 840 |
| TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAGGC GCTGAATGAT | 900 |
| ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG | 960 |
| GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCCTGAGG TGTTTGGCAA GCCGCAGTAC | 1020 |
| CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAA AGCACTGAGC | 1080 |
| AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC | 1140 |
| ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC | 1200 |
| GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA | 1260 |
| CTTGGCAAGC TGGGCGCGGC TTAAGCTT | 1288 |

- 89 -

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 341 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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20          25
Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
35          40          45
Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
50          55          60
Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
65          70          75          80
Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
85          90          95
Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
100          105          110
Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
115          120          125
Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
130          135          140
Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145          150          155          160
Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
165          170          175
Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
180          185          190
Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
195          200          205
Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
210          215          220
Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
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Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
245          250          255

```

- 90 -

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
 260 265 270
 Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
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 Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
 290 295 300
 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
 305 310 315 320
 Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
 325 330 335
 Asn Gln Ala Ala Ala
 340

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1026 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

| | |
|--|-----|
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| GTACGTCTCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC | 120 |
| GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA | 180 |
| AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC | 240 |
| ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG | 300 |
| GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC | 360 |
| AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC | 420 |
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| AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC | 540 |
| GAAACGGCTG CGTTCCGTTT GGCACGCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG | 600 |
| AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC | 660 |
| AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC | 720 |
| GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA | 780 |
| TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCAGAC CCGTACGTCG | 840 |

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GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG 900
 GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT 960
 GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA 1020
 GCCTGA 1026

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 344 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
 1 5 10 15
 Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
 20 25 30
 Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
 35 40 45
 Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
 50 55 60
 Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
 65 70 75 80
 Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
 85 90 95
 Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
 100 105 110
 Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
 115 120 125
 Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
 130 135 140
 Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
 145 150 155 160
 Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
 165 170 175
 Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
 180 185 190

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Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
 195 200 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
 210 215 220

Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
 225 230 235 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
 245 250 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln
 260 265 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
 275 280 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
 290 295 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
 305 310 315 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
 325 330 335

Gln Ser Thr Ser Thr Gln Pro Met
 340

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1035 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

| | |
|---|-----|
| ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC | 60 |
| AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC | 120 |
| GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC | 180 |
| GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC | 240 |
| AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC | 300 |
| GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA | 360 |
| GACCTGGTGA AGCTGCTGAA GCGGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG | 420 |
| GGCAACGGCG TGGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC | 480 |

| | | | | | | |
|------------|------------|------------|------------|------------|------------|------|
| GAAGCGCTGC | AGGAGATCGA | GCAGATCCTC | GCCCAGCTCG | GCGGCGGCGG | TGCTGGCGCC | 540 |
| GGCGGCGCGG | GTGGCGGTGT | CGGCGGTGCT | GGTGGCGCGG | ATGGCGGCTC | CGGTGCGGGT | 600 |
| GGCGCAGGCG | GTGCGAACGG | CGCCGACGGC | GGCAATGGCG | TGAACGGCAA | CCAGGCGAAC | 660 |
| GGCCCGCAGA | ACGCAGGCGA | TGTCAACGGT | GCCAACGGCG | CGGATGACGG | CAGCGAAGAC | 720 |
| CAGGGCGGCC | TCACCGGCGT | GCTGCAAAAG | CTGATGAAGA | TCCTGAACGC | GCTGGTGCAG | 780 |
| ATGATGCAGC | AAGGCGGCCT | CGGCGGCGGC | AACCAGGCGC | AGGGCGGCTC | GAAGGGTGCC | 840 |
| GGCAACGCCT | CGCCGGCTTC | CGGCGCGAAC | CCGGGCGCGA | ACCAGCCCGG | TTCGGCGGAT | 900 |
| GATCAATCGT | CCGGCCAGAA | CAATCTGCAA | TCCCAGATCA | TGGATGTGGT | GAAGGAGGTC | 960 |
| GTCCAGATCC | TGCAGCAGAT | GCTGGCGGCG | CAGAACGGCG | GCAGCCAGCA | GTCCACCTCG | 1020 |
| ACGCAGCCGA | TGTAA | | | | | 1035 |

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
1 5 10 15
Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
20 25

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
1 5 10 15
Leu Leu Ala Met
20

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WHAT IS CLAIMED:

1. A method of enhancing growth in plants comprising:

applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to a plant or plant seed under conditions effective to enhance growth of the plant or plants grown from the plant seed.

2. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a pathogen selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and mixtures thereof.

3. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia chrysanthemi*.

4. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia amylovora*.

5. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas syringae*.

6. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas solanacearum*.

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7. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Xanthomonas campestris*.

8. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to a *Phytophthora* species.

9. A method according to claim 1, wherein the plant is selected from the group consisting of dicots and monocots.

10. A method according to claim 9, wherein the plant is selected from the group consisting of rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

11. A method according to claim 9, wherein the plant is selected from the group consisting of rose, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

12. A method according to claim 1, wherein plants are treated during said applying which is carried out by spraying, injection, or leaf abrasion at a time proximate to when said applying takes place.

13. A method according to claim 1, wherein plant seeds are treated during said applying which is

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carried out by spraying, injection, coating, dusting, or immersion.

14. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein is applied to plants or plant seeds as a composition further comprising a carrier.

15. A method according to claim 14, wherein the carrier is selected from the group consisting of water, aqueous solutions, slurries, and powders.

16. A method according to claim 14, wherein the composition contains greater than 0.5 nM of the hypersensitive response elicitor polypeptide or protein.

17. A method according to claim 14, wherein the composition further contains additives selected from the group consisting of fertilizer, insecticide, fungicide, nematocide, and mixtures thereof.

18. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein is in isolated form.

19. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein is applied as bacteria which do not cause disease and are transformed with a gene encoding the hypersensitive response elicitor polypeptide or protein.

20. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein is applied as bacteria which cause disease in some plant species, but not in those subjected to said applying, and

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contain a gene encoding the hypersensitive response elicitor polypeptide or protein.

21. A method according to claim 1, wherein said applying causes infiltration of the polypeptide or protein into the plant.

22. A method according to claim 1, wherein said applying effects increased plant height.

23. A method according to claim 22, wherein plants are treated during said applying.

24. A method according to claim 22, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and propagating the plants from the seeds planted in the soil.

25. A method according to claim 1, wherein plant seeds are treated during said applying to increase plant seed quantities which germinate, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and
propagating plants from the seeds planted in the soil.

26. A method according to claim 1, wherein said applying effects greater yield.

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27. A method according to claim 26, wherein plants are treated during said applying.

28. A method according to claim 26, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and propagating plants from the seeds planted in the soil.

29. A method according to claim 1, wherein said applying effects earlier germination.

30. A method according to claim 29, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and propagating plants from the seeds planted in the soil.

31. A method according to claim 29, wherein said applying effects earlier maturation.

32. A method according to claim 31, wherein plants are treated during said applying.

33. A method according to claim 31, wherein plant seeds are treated during said applying, said method further comprising:

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planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and propagating plants from the seeds planted in the soil.

34. A method according to claim 1, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and propagating plants from the seeds planted in the soil.

35. A method according to claim 34 further comprising:

applying the hypersensitive response elicitor protein or polypeptide in a non-infectious form to the propagated plants to enhance growth further.

36. A method according to claim 1, wherein said applying effects earlier fruit and plant coloration.

37. A method according to claim 36, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and propagating plants from the seeds planted in the soil.

38. A method of enhancing growth in plants comprising:

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providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and

growing the transgenic plants or transgenic plants produced from the transgenic plant seeds under conditions effective to enhance plant growth.

39. A method according to claim 38, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a pathogen selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and mixtures thereof.

40. A method according to claim 39, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia chrysanthemi*.

41. A method according to claim 39, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia amylovora*.

42. A method according to claim 39, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas syringae*.

43. A method according to claim 39, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas solanacearum*.

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44. A method according to claim 39, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Xanthomonas campestris*.

45. A method according to claim 39, wherein the hypersensitive response eliciting polypeptide or protein corresponds to that derived from a *Phytophthora* species.

46. A method according to claim 38, wherein the plant is selected from the group consisting of dicots and monocots.

47. A method according to claim 46, wherein the plant is selected from the group consisting of rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

48. A method according to claim 46, wherein the plant is selected from the group consisting of rose, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

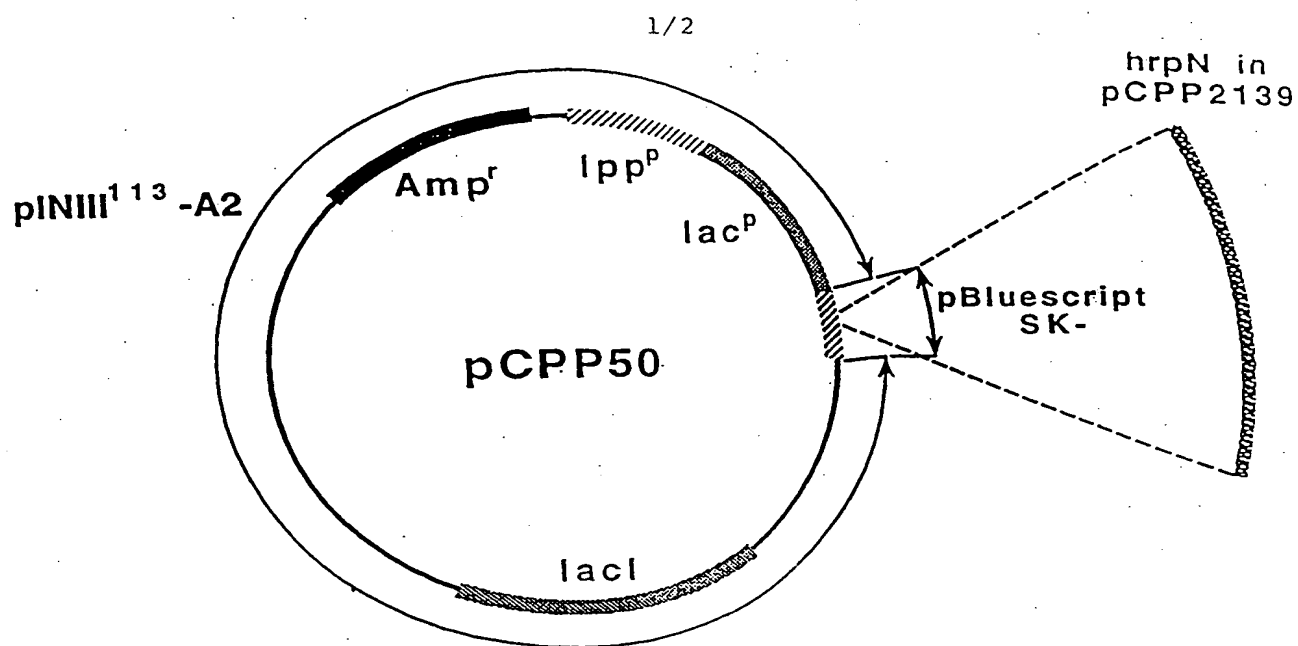
49. A method according to claim 38, wherein a transgenic plant is provided.

50. A method according to claim 38, wherein a transgenic plant seed is provided.

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51. A method according to claim 38 further comprising:

applying the hypersensitive response elicitor polypeptide or protein to the propagated plants to enhance growth of the plant.



Part of polylinker from pBluescript SK- (XbaI to HindIII).
From Stratagene, La Jolla, CA.

FIGURE 1

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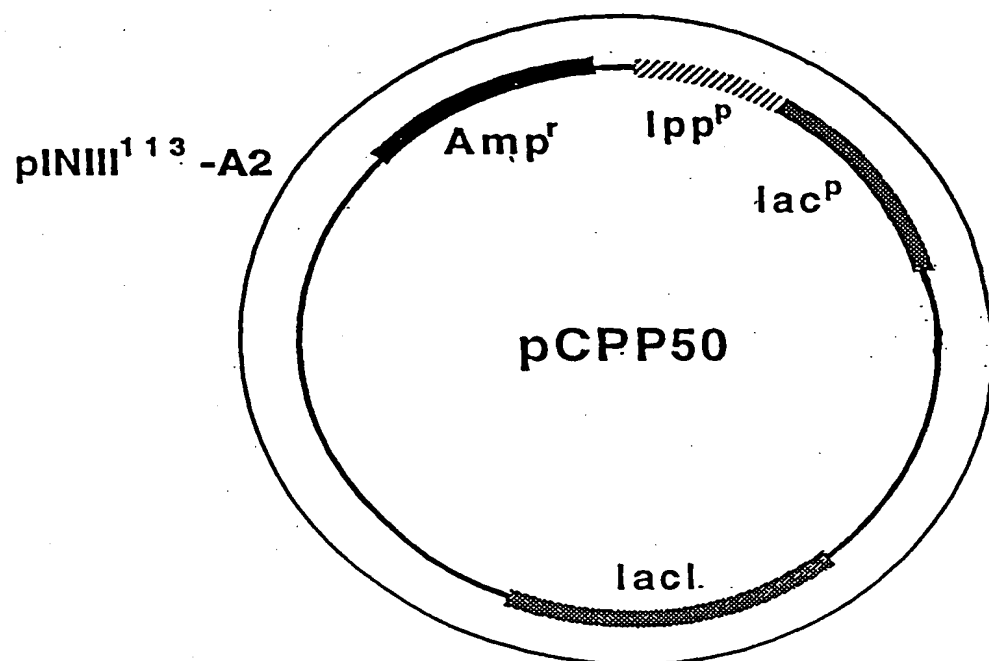


FIGURE 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01507

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 15/03, 15/05; A01N 13/00; A61K 39/00 1/11 1/15; A01H 4/00

US CL : 47/87; 435/252.3; 800/205

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 47/87; 435/252.3; 800/205

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Biosis, Medline, Wpids, NCBI, Agricola

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| Y | WEI et al. Harpin Elicitor of the Hypersensitive Response Produced by the Plant Pathogen Erwinia Amylovora. Science. July 1992, Vol. 257, pages 85-88. See entire reference. | 1-18, 21-37 |
| X | BURR et al. Increase Potato Yields by Treatment of Seed Pieces with Specific Strains of Pseudomonas fluorescens and P. putida. Phytopathology. September 1978, Vol. 68, pages, 1377-1383. See entire reference | 1-2, 9-10-18, 21-37 |
| Y | | ----- 1-18, 21-37 |
| Y | KLOEPPER et al. Enhanced Plant Growth by Siderophores Produced by Plant Growth-Promoting Rhizobacteria. Nature. 28 August 1980, Vol. 286, 885-886. See entire reference. | 1-18, 21-37 |



Further documents are listed in the continuation of Box C.



See patent family annex.

| | |
|---|--|
| * Special categories of cited documents: | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| *A* document defining the general state of the art which is not considered to be of particular relevance | *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| *B* earlier document published on or after the international filing date | *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *A* document member of the same patent family |
| *O* document referring to an oral disclosure, use, exhibition or other means | |
| *P* document published prior to the international filing date but later than the priority date claimed | |

Date of the actual completion of the international search

09 MAY 1998

Date of mailing of the international search report

19 JUN 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

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Authorized officer

Ousama M-Faiz Zaghmout

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01507

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-18,21-37
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/01507

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not linked as to form a single inventive concept under PCT Rule 13.1.

Group I. Claims 1-18, 21-37 are drawn to a method of enhancing growth in plants by applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to plants or seeds, classified in class 47, subclass 87 for example.

Group II. Claims 19-20 are drawn to a method wherein the hypersensitive response elicitor polypeptide or protein is applied as bacteria, classified in class 435, subclass 252.3 for example.

Group III. Claims 38-51 are drawn to a method for enhancing the growth of plant by producing transgenic or plant seed by transformation with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein, classified in class 800, subclass 205 for example.

The inventions listed as groups I, II and III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The invention of group I, drawn to a first process of making a first product, involves a method of enhancing the growth in plants by applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to plants or seeds not required by the groups II and III. The use of bacteria of invention in group II or transgenic plants of invention in group III are not required by the invention of group I.

The invention of group II involves a method wherein the hypersensitive response elicitor polypeptide or protein is applied as bacteria, not required by groups I where the response can be induced by protein which can be chemically synthesized, or by invention of group III where transgenic plants instead of bacteria.

The invention of group III to a method for enhancing the growth of plant by producing transgenic or plant seed by transformation with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein, not required by group I and II for the reasons specified above.